THE

BOTANICAL GAZETTE

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ERRATA

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P. 312 and throughout article, for *"lessigniana"* read *"lessigiana"*

Vol. XCI

P. 237, lines 23 and 29, for *"adnatum"* read *"adnata"*

P. 260, line 3 from bottom, for "content (right), and another" read "content;
(right) another"

IMMUNOLOGICAL RELATIONSHIP OF WHEATS RESISTANT AND SUSCEPTIBLE TO PUCCINIA RUBIGO-VERA TRITICINA[1]

ALBERT EDWARD EDGECOMBE

Introduction

It is generally assumed that each individual organism is characterized by its specific protoplasm, and that degree of similarity of protoplasm is a criterion of genetic relationship. While the carbohydrates and fats (REICHERT **22, 23**) that occur as inclusions, or that enter into composition of the protoplasm of an individual display considerable specificity, the proteins seem to be the main factors in biological specificity (WELLS **28**). If one postulates evolution of organisms then one must postulate also an evolution of proteins. This may have involved new combinations of the 20 or so amino acids that are found to make up the protein molecule. ABDERHALDEN has calculated that 20 different amino acids could make 2, 432, 902, 008, 176, 640,000 different compounds, without including those that might be made by varying the proportion of the different amino acids in a single protein (WELLS **28**), and thus enable proteins to display variability of an order exhibited by organisms themselves. Immunological technique supplies a means of detecting protein identities, differences, and relationships which lie far beyond the range of the usual chemical technique. WELLS (**29**) points out that while the

[1] Space for the animals and experimental work was provided through the courtesy of Dr. W. H. TALIAFERRO, of the Department of Hygiene and Bacteriology.

biuret reaction will detect proteins in a dilution of 1:1000 or so, the precipitin test and the complement fixation test will detect proteins in dilutions of 1:10,000–100,000 and 1:1,000,000 respectively. Moreover, while the biuret test merely indicates the presence of a protein, the immunological test may tell which specific protein is present.

Immunological, or serological, technique exploits the fact that free proteins, such as egg white, and cells, such as bacterial thalli, apparently because of their proteins, can act as immunizing antigens when injected into the blood stream of suitable animals in proper dosages at proper intervals, engendering the formation of more or less specific antibodies. When these antibodies are brought into contact under suitable conditions with the protein used as immunizing antigen, or with other proteins called test antigens, they lead to specific reactions limited to, or more pronounced between, the immunizing antigen and its homologous antibody. Reactions with other proteins and the intensity of the reaction indicate more or less relationship between the protein of the immunizing antigen and of the test antigens. It appears that while the antigenic capacity of a protein depends upon the entire large colloidal molecular structure, its specificity seems to reside in certain of the radicals of the molecule. If a protein molecule possesses more than one reactive radical it may show more than one specific immunological reaction. Consequently group reactions exhibited by complex antigens from biologically related species may depend either upon the presence in these antigens of both common and specific proteins, or upon the presence in different proteins of common and specific reactive radicals. Even a single group within a radical may determine the specific immunological behavior of the entire molecule. Both the location and the character of the specific group are factors in determining specificity of the molecule. Specificity is apparently always a quantitative matter, reaching its maximum when the antigen is reacting with an antibody produced by immunizing with an identical antigen (WELLS 28).

It has been shown that the globulin or prolamine fractions of plant proteins are highly potent and specific as antigens. Some of the most convincing chemical work of immunology was done with plant pro-

teins. OSBORNE (19) prepared various plant proteins as purely as possible, which WELLS (18) used in immunological tests, arriving at the conclusion that immunological specificity is dependent upon chemical individuality of the antigen. A study of the globulins of beans by JONES and WELLS (29) has demonstrated that the mung and adzuki beans are closely related to each other but distinct from other beans. LEWIS and associates (12) have demonstrated that the prolamines of emmer, einkorn, spelt, and durum wheats are closely related to gliadin and glutenin of *T. vulgare*, while those of teosinte and kafir are closely related to zein from *Zea mays*. No reactions were obtained between the proteins of the corn and wheat groups.

Serological technique has been used, not only to establish the identity of antigens or antibodies, as in detection of adulteration in meats, in flour, or meal of plant origin, or in bacterial infections, but it has also been used in phylogenetic studies, first for animals by NUTTALL (15), and later for plants in a less precise way by MEZ and his students (13). In the main the conclusions are in agreement with those based on comparative morphology, and when they are not, the serological evidence at times is given preference. The work of MEZ, however, has not been received favorably. MEZ (14) pointed out that when a serological test is made in phylogenetic studies, the protein reaction is in fact giving a clue to the relationship of the idioplasm of the organisms, and postulated that because of the great rôle of the nucleus in heredity, immunological tests give a clue to relationship of the nuclear proteins. If this be true, then on account of the rôle of chromosomes in heredity and phylogenetic relationship, it is possible that serological technique may be a means of studying the relationship of the proteins of the chromosomes.

It seems natural that serological technique should be applied as an aid in determining the systematic relations of economic plants in breeding work, and that geneticists have given heed to it as a possible means for determining invisible characters. ZADE (30) applied serological technique (specifically, the precipitin test) to determine the systematic relationships of oats and wheats. He pointed out that the morphological affinity as determined by appearance, and sexual affinity as determined by compatability in hybridization, are not reliable criteria in every instance. He found that the serolog-

ical relationships of oats and wheats are in good agreement with the
relationships determined by morphological and breeding criteria.
VAVILOV (27) pointed out that the groupings determined serologi-
cally by ZADE are in essential agreement with the groupings ob-
tained when he used the criteria of resistance-susceptibility of wheats
to *Puccinia rubigo-vera triticina* (red rust), and of anatomical and
morphological characters in genetic and systematic study of these
plants.

SAX (21, 24), in summarizing extensive cytological work by him-
self and others with wheats, points out that there is a striking asso-
ciation between chromosome number and morphological and other
characters, such as adaptability to environmental conditions, disease
resistance, quality of grain, and economic value. Chromosome num-
ber also plays a powerful rôle in sterility and genetic relationships.
The einkorn group (haploid, 7 chromosomes) contains few varieties
and is of little economic value. It is highly resistant to rust. The
emmer group (haploid, 14 chromosomes) contains about 150 varieties
and is of considerable value in semiarid regions. Its members are
relatively resistant to rust. The vulgare group (haploid, 21 chromo-
somes) contains 500 varieties which are very adaptable, have proper
quality of gluten for light bread, and are generally susceptible to
rust. The increase in chromosome number and correlated increase
in variability may be attributed to chromosome reduplication or in-
crease in chromosome number through crossing, thereby offering
opportunity for mutations to occur, and permitting the effect of a
greater number of genetic factors (SAX 24).

NELSON and BIRKELAND (16) used immunological technique to
differentiate varieties within species, and demonstrated that the
precipitin test, when conducted with the purified globulin fraction of
wheat proteins, is of value to the geneticist in selecting hybrids for
desired invisible characters, such as resistance of wheats to *Puccinia
graminis tritici*. When any one variety is used as a standard, others
can be classified serologically, the wheats showing the greatest num-
ber of genetic factors in common showing the closest relationship
serologically.

Immunological technique has also been used to determine com-
patibility of individuals for grafting (DONTCHO 4, GREEN 5).

Investigation

This research was undertaken, at the suggestion of Professor
GEORGE K. K. LINK, to determine the serological relationship of
species, varieties, and hybrids, selected on the basis of their resist-
ance to red rust, of the einkorn, emmer, and vulgare groups of
wheats, and also to determine whether a parallelism or correlation
exists between their ranking as determined by (a) immunological
methods, (b) their chromosome number, and (c) their reaction to
Puccinia rubigo-vera (Erikss) *triticina* (Mains and Jackson). The
plan was to use representatives of the highly resistant einkorn
(haploid, 7 chromosomes) group; representatives of the resistant
emmer (haploid, 14 chromosomes) group; resistant and susceptible
representatives of *T. dicoccum* (haploid, 14 chromosomes) which by
some is taken to be the polymorphic progenitor of the susceptible
and immune species of *T. vulgare* and *T. durum* (VAVILOV 26); and
susceptible, slightly resistant and resistant representatives of the
T. vulgare (haploid, 21 chromosomes) group.

Because of the suspected but moot rôle of *Aegilops* species in the
formation of the vulgare group, it was desirable to include a repre-
sentative of this genus. PERCIVAL (20) contends that the vulgare
wheats have arisen from a cross of *Aegilops* and a wheat of the em-
mer group, the *Aegilops* contributing, among other characters, sus-
ceptibility to rust. According to SAX (24), crosses of *A. cylindrica*
and *T. vulgare* indicate that the two genera have one set of chromo-
somes in common. GAINES (21), on the basis of finding no paired
chromosomes at reduction in crosses of *A. ovata* and emmer, suggests
that there are 4 sets of chromosomes in the genera *Triticum* and
Aegilops: einkorn containing chromosome groups *A* or *B;* emmer,
A and *B; Aegilops, C* and *D;* and vulgare, *A, B,* and *C.* SAX (21)
states that recent work of TSCHERMAK and BLEIER does not support
this view. A fertile homozygous segregate from a cross of *A. ovata*
and an emmer wheat was obtained. The F_1 hybrid had 28 pairs of
chromosomes, due to doubling of the chromosome number in the
first division of the fertilized egg.

Shortly after formulation of the early plans, Dr. E. B. MAINS,
then at Purdue University Agricultural Experiment Station, was
approached for suggestions as to suitable material. Dr. MAINS kind-

ly suggested that it would be well to include representatives of the vulgare group which show one type of response to rust in the seedling stage and another in maturity, and also a hybrid of the emmer (14) group and the vulgare (21) group which showed the resistance of the emmer group. This was of interest because SAX (24) apparently questions whether resistance can be transferred from varieties of *T. durum* to *T. vulgare* by crossing, stating that resistance frequently is dependent on the physiological condition of the hybrid. HAYES, however, points out that THOMPSON (21) was able to transfer resistance from durums to common wheats, and that he and his associates accomplished similar results. MCFADDEN (21) crossed *T. dicoccum*, resistant, a 14-chromosome wheat, with Marquis, a 21-chromosome wheat, and obtained a 21-chromosome plant, Hope, which is resistant.

The original plan had to be abandoned because it was found impractical to procure the desired forms[2] within each group, or to obtain sufficient quantity of seed to make the project feasible. From 500 to 1000 gm. of seed of each sample was needed to undertake the work, and it was therefore necessary to select those varieties of which a sufficient quantity of material was available.

The nine forms of wheat finally used in this research fall into three categories, based on their chromosome count, namely, the einkorn (7[3] haploid number), the emmer (14 haploid number), and the vulgare (21 haploid number) groups. Only one form was used in the einkorn group, and this was the most resistant of those tested. It is a double einkorn. In the emmer group three forms were used, one durum, and two emmers, and these were all leaf-rust resistant. Five forms were tested in the vulgare group, and these, the common wheat forms, varied from highly resistant to very susceptible to rust.

It was particularly unfortunate that but one form was procurable in the einkorn group, that no leaf-rust susceptible form was available

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[2] In view of the fact that this study involved the use of both species and varieties, the term "form" is used to stand for species, varieties, or both. This is done to avoid repetition of the phrase "species and varieties."

[3] In the remainder of this paper the numbers (7), (14), and (21) indicate that the forms belong to the chromosome group represented by the numbers in brackets.

in the emmer group, and that no representative of *Aegilops* was obtained. These facts in themselves are sufficient to diminish somewhat the chances of securing the most striking results possible. Fortunately, however, through Dr. Mains' kindness, a resistant hybrid (Hope, 21) of the emmer (14) and vulgare (21) groups was obtained, as well as a representative of *T. vulgare* which is very resistant as it matures, but which is susceptible to a number of physiological forms in its seedling stage.

TABLE I

Group	Form	Common name	No. of wheat and designation	Resistance rating
1. Einkorn (7)	T. monococcum.	Einkorn	1* Ei	Very resistant
2. Emmer (14)	T. durum.......	Durum	3320 D	Resistant
	T. dicoccum....	Vernal emmer	1524 Em$_1$	Resistant
	T. dicoccum....	Emmer	293 Em$_2$	Resistant
3. Vulgare (21)	Hope..........	Common wheat	8178 Co$_4$	Highly resistant
	Marquis........	Common wheat	3641 Co$_3$	Slightly resistant
	T. vulgare......	Common wheat	2036 Co$_2$	Resistant in maturity; susceptible in seedling stage
	T. vulgare......	Michigan amber	29 Co$_1$	Susceptible
	T. compactum..	Little club	4066 Cl	Susceptible

* Number under which the wheat was received from Dr. Mains.

Table I summarizes the forms of wheat used in this investigation; the number under which they were received from Dr. Mains; their grouping according to chromosome number; and their ranking on the basis of resistance-susceptibility to red leaf-rust as determined by inoculation experiments. Consequently, this research repeats in part Zade's experiment, differing from it in that (a) genetically indexed species, varieties, and forms were used; and (b) purified globulin fractions were used (as in the Nelson and Birkeland (16) experiments) rather than the crude protein extracts used by Zade. In this way it was hoped to differentiate between varieties which Zade found difficult and impossible for oats when the varieties are of com-

mon descent. The paper is more comprehensive than that of NELSON and BIRKELAND, in that it involves representatives of the three great chromosome groups, and differs in that forms of wheat were selected on the basis of their resistance to *Puccinia rubigo-vera triticina*. Absorption tests were not made, however, as in the tests of NELSON and BIRKELAND. This would have added materially to the interest and significance of the results.

The globulin fractions of the protein of the grain of the varieties tested were obtained pure by electrodialysis, and then used as immunizing antigens by injection into rabbits. The precipitin test was used in which the criterion of reaction is the formation of a specific precipitate when immunizing antigen and antibody are brought into contact. The gross precipitate method first used was later abandoned for the more precise ring test, in which the lighter antigen solution is carefully layered in fine capillary tubes on the heavier antiserum in proper dilutions, and the precipitate appears as a ring at the interphase.

Method

The method involved four phases: grinding the wheat grains, dialysis of the extracted globulins, animal immunization, and serum titrations. From 400 to 1000 gm. of each form of wheat seed was put first through a coarse and finally through a fine mill. In every case the final product was fine enough to pass through a standard 100-mesh sieve.

DIALYSIS

The flour was first extracted in 300 gm. lots over a period of 12–18 hours in a 10 per cent sodium chloride solution, allowing 1 gm. of flour to 10 cc. of salt solution. At the end of this time the salt extract of soluble globulin was siphoned off, filtered through two thicknesses of fine toweling, and the filtrate dialyzed for 8–12 hours in an electrodialysis apparatus similar to that described by LOCKE and HIRSCH (9).

Two collodion bags of diameter (4.5 cm.) and length (16 cm.) were each fitted with rubber stoppers of the same bore, with a long inlet and short outlet of glass tubing for intake and output of distilled water. The stoppers were fitted with carbon poles. The col-

lodion bags were made from an 8 per cent solution of celloidin in a solution of equal parts of absolute alcohol and pure ether (BROWN 2). These bags were molded in the inside of large glass tubes according to the method detailed by HAWK (8). After the ether had evaporated the bags were removed with water, dried, and then adjusted for suitable permeability for 12 hours in an 85 per cent alcohol solution (BROWN 3). The bags were not allowed to dry after they had once been adjusted for permeability in alcohol. As soon as the bags were ready they were tied to closely fitting rubber stoppers and sealed fast with collodion. The inlet and outlet glass tubing and the carbon pole all had water-tight fittings. A pair of such bags was suspended and immersed in a museum jar of three or four liter capacity, containing sodium chloride extract of globulin. Iron clamps attached to the rubber stoppers and fastened to iron stands held the bags in place. A continuous flow of water from a single source was maintained through both bags, with just sufficient pressure to keep them distended.

The energy for electrodialysis was supplied from a direct current giving 110 volts in the primary circuit. By the introduction of a sliding rheostat, a secondary circuit was maneuvered which gave 60 volts. The dialyzer was started at 25 volts and gradually raised to 50 volts as the dialysis neared completion. A two-way switch was introduced into the set-up leading to the dialyzing apparatus, to afford a means for periodically changing the direction of flow of the current through the solution to prevent polarization.

After the supernatant liquid was siphoned off, the globulin precipitate so obtained was redissolved in 10 per cent sodium chloride solution, and diluted to 5 per cent and again dialyzed. A final purification of the globulin precipitate was made by dialysis from physiological salt solution. The purified globulin precipitate obtained after the third dialysis was filtered through hard filter paper, using a Büchner funnel and suction pump. The precipitate was washed on the filter paper with distilled water, and dried partially with the suction pump, and finally at 25° C. It was then pulverized in a sterile agate mortar and stored in glass vials. Each variety of wheat was treated in the same way.

IMMUNIZATION

Rabbits weighing approximately five pounds each were used for immunization. Each antigen was prepared in duplicate and six intravenous injections were given each animal at 4-day intervals. The immunizing antigen was dissolved in physiological salt solution (0.85%) to give a concentration of 0.005 gm. per cubic centimeter, and 1 cc. for the first injection and 2 cc. each for the other five injections were given as the immunizing dose. Before injection the immunizing antigen was just neutralized by the addition of N/20 NaOH.

Enough antigen material was prepared at one time for three injections, and this was held in the refrigerator for the interval of 8 days between the first and third injections. A second lot of antigen material was similarly prepared and stored at icebox temperature for the three other injections. Nine days after the sixth injection the rabbits were bled from the heart, and 25–30 cc. of arterial blood was drawn and placed in sterile flasks. When the clot formed the clear serum was transferred aseptically to sterile test-tubes and held in the refrigerator until such time as the precipitin titrations were run.

Two normal rabbits were held as controls and these were bled at the same time. Three days after the rabbits were bled, they was again injected with 2 cc. of 0.005 gm. per cubic centimeter of freshly prepared antigen and then bled on the eleventh day following the eighth and final injection. The second lot of serum was drawn aseptically from the heart, prepared, stored, and used in the final precipitin titrations. The results of the last test alone are reported in this paper.

Precipitin tests

During the second period of animal immunization, preliminary tests were made with the antiserum obtained at the end of the first period of immunization. All data recorded in this paper, however, are the results of the ring precipitin test made from titrations with the antiserum obtained after the second period of immunization.

Following NELSON and BIRKELAND (16), the mass precipitin test was first tried in these preliminary investigations. In this method regular Wassermann agglutination tubes were used and 0.5 cc. of diluted antiserum was pipetted into the tube to which 1 cc. of antigen

was afterwards added. A thorough mixing of the antigen-antiserum was accomplished by vigorously shaking the tubes, which were then allowed to stand at room temperature for one hour, read, and then incubated at 37° for 3 hours, again read at the end of the fourth hour, and held in the icebox overnight, when a final reading was taken for the quantity of precipitate deposited.

In all the mass precipitin titrations the test antigens were prepared at a concentration of 0.0005 gm. per cubic centimeter, and adjusted to neutrality with a solution of twentieth normal sodium hydroxide, the original pH of the antigen being 5.8. The antiserum was diluted with physiological salt solutions so as to give dilutions of 1–10, 1–20, 1–40, 1–80, 1–160, 1–320, 1–640, 1–1280, and 1–2560, and tested in these dilutions in every instance against its homologous immunizing and the heterologous test antigens. There was no apparent increase in precipitation after the first 4 hours, and hence the tubes were no longer held at icebox temperature. The small amount of precipitate and the difficulty of estimating quantitatively the amount of precipitate formed in the several dilutions made the task of reading the tubes difficult and the results unsatisfactory. In fact the results were so disappointing and conflicting that the method was abandoned after a fair trial with duplicate antiserum had been made.

In the final preliminary investigations, the ring precipitin test was used. In this test regular (bore 3 mm.) precipitin tubes were used. The test antigen was prepared in the concentrations already recorded and the antiserum in the same dilution as stated before. Enough antiserum was pipetted into the tube with a micropipette to fill it about one-third full, and then by the same procedure an equal amount of test antigen was carefully layered on to the antiserum. Several methods of incubating were tested, at icebox temperature, in a water-bath at 37° C., and also at room temperature. It was found that the best rings were formed within the first hour at room temperature; consequently all subsequent readings were made one hour after the layering of the antigen-antiserum, and then again as a check at the end of the fourth hour. It was further observed that the best results were obtainable when the test antigen was adjusted to pH 7 or to a pH slightly in the alkaline range.

The ring precipitin test gave definite results and indicated that the method was feasible for a study of the nine wheats. With these preliminary tests as a background, a more exhaustive and comprehensive study of the nine antigens was undertaken, and they were tested against their homologous and heterologous antisera obtained from the second immunization. This time the work was run in relays, three antisera in various dilutions against the nine antigens, until the lot of eighteen antisera were tested, always using the ring precipitin test.

TABLE II

TITRES OF PRECIPITIN TESTS

VERSUS ANTIGEN	ANTISERUM								
	Ei	D	Em₁	Em₂	Co₄	Co₃	Co₂	Co₁	Cl
Ei.............	[7]*	4	3	3	2–3	2	2	1–2	1
D.............	4	[7]	4	4	3–4	3	2–3	3	2
Em₁............	3	4	[7]	3	3	2	3	2–3	2–3
Em₂............	4	4	4	[6]	3–4	3	2	2	1–2
Co₄............	3	4	3–4	3	[6]	2–3	3	2	3
Co₃............	2	2	3	2–3	2–3	[5]	3	2	3
Co₂............	1–2	2–3	3	1–2	2	3	[6]	2	4
Co₁............	1	2	2	1–2	2	2–3	3	[5]	4
Cl.............	1	2	2	1–2	1–3	3	2	4	[7]

* The numbers recorded represent the titre of the test. Thus 1 represents a maximum titre in dilution 1–10; 7, in dilution 1–640; 4, in dilution 1–80; 2–3, in dilution ranging from 1–20 to 1–40, a definite decision between them being impossible. The numbers in brackets indicate the titres of the homologous reactions. The ranking of the antigens is arbitrary but on the basis of their chromosome numbers.

As a check, titrations of antigen-antiserum were run in which the antigen was diluted. The results were less definite than with the titrations of antigen-antiserum in which the antiserum was diluted. Titrations of antigens against normal serums, antigens against saline, and antiserums against saline all were uniformly negative.

The summarized data of table II show only the results from a single animal, since the duplicate tests with the second animal did not affect the final data to any significant extent. The numbers recorded in the table represent titres, titre being here used in the sense of highest dilution in which ring formation could be observed. At serum dilutions of from 1–80 to 1–160 the heaviest rings were obtained in the homologous reading. Table III shows the ranking of

the antisera on the basis of their titres and on the basis of their chromosome group relations, while table IV shows their ranking on the basis of their titres and their resistance-susceptibility relations.

TABLE III

RANK OF ANTISERUMS ON BASIS OF TITRES AND CHROMOSOME NUMBERS

Chromosome number	Versus antigen	Antiserum								
(7)........	Ei	Ei*	$[D$*	Em_1	$Em_2]$	$[Co_4$	Co_3	Co_2	Co_1	$Cl]$
(14).......	D	$[D$	Em_1	$Em_2]$	Ei	$[Co_4$	Co_3	Co_1	Co_2	$Cl]$
	Em_1	$[Em_1$	D	$Em_2]$	Ei	$[Co_4$	Co_2	Co_1	Cl	$Co_3]$
	Em_2	$[Em_2$	D	$Em_1]$	Ei	$[Co_4$	Co_3	Co_2	Co_1	$Cl]$
(21).......	Co_4	Co_4	$[D$	Em_1	$Em_2]$	Ei	$[Co_2$	Cl	Co_3	$Co_1]$
	Co_3	$[Co_3$	Co_2	$Cl]$	Em_1	Co_4	Em_2	Co_1	D	Ei
	Co_2	$[Co_2$	Cl	$Co_3]$	Em_2	Co_4	D	Co_1	Em_1	Ei
	Co_1	$[Co_1$	Cl	Co_2	Co_3	$Co_4]$	$[D$	Em_1	$Em_2]$	Ei
	Cl	$[Cl$	Co_1	Co_2	Co_3	$Co_4]$	$[D$	Em_1	$Em_2]$	Ei
Ranking on basis of reaction........		1	2	3	4	5	6	7	8	9

* Brackets indicate that members of a chromosome group form a serological group. Vertical order of arranging the antigens is that used in table II; the horizontal is according to titre.

TABLE IV

RANK OF ANTISERUMS ON BASIS OF TITRES AND RESISTANCE-SUSCEPTIBILITY REACTIONS

Host reaction	Versus antigen	Antiserum								
Resistant..........	Ei	Ei	D	Em_1	Em_2	Co_4	Co_3	Co_2	Co_1	Cl
	D	D	Ei	Em_1	Em_2	Co_4	Co_3	$[Co_1]$	Co_2	Cl
	Em_1	Em_1	D	Em_2	Ei	Co_4	Co_2	Co_1	Cl	$[Co_3]$
	Em_2	Em_2	D	Em_1	Ei	Co_4	Co_3	Co_2	Co_1	Cl
	Co_4	Co_4	D	Em_1	Em_2	Ei	Co_2	$[Cl]$	Co_3	Co_1
Resistant but susceptible in seedling stage.............	Co_2	Co_2	Cl	Co_3	Em_1	D	Co_4	$[Co_1]$	Em_2	Ei
Slightly resistant....	Co_3	Co_3	Co_2	Cl	Em_1	Co_4	Em_2	$[Co_1]$	D	Ei
Susceptible.........	Co_1	Co_1	Cl	Co_2	Co_3	Co_4	D	Em_1	Em_2	Ei
	Cl	Cl	Co_1	$[Co_2]$	Co_3	Co_4	D	Em_1	Em_2	Ei
Ranking on basis of reaction..........		1	2	3	4	5	6	7	8	9

* Brackets indicate deviations from ideal ranking, ranging from invariably very resistant to resistant through variably or slightly resistant to invariably susceptible; or from invariably susceptible through slightly or variably resistant to invariably resistant or very resistant.

Discussion

Table II shows that in each instance the homologous antigen and antiserum gave the highest titre, indicating specificity of the reaction and individuality of each globulin. On the other hand, the group reaction of every antigen with every antiserum indicated that considerable relationship exists between the globulins of all the forms tested, or that all forms have some globulins in common. Tables II–IV show that serologically the members of the 14 chromosome group are closely related, and that they are related to $T.$ $monococcum$ (7). The members of the 21 group do not show as close a relationship among one another as do those of the 14 group. Some of them are more closely related to members of the 7 or 14 groups than they are to one another. For example, the highly resistant CO_4 (21) is more closely related to $T.$ $monococcum$ (7) and to D, Em_1, and Em_2 (14) than to CO_3 and CO_1 (21); CO_3 (21) is more closely related to Em_1 (4) than it is to CO_1 (21); and CO_2 (21) is more closely related to Em_1 (14) than to CO_4. On the other hand, CO_1 and Cl (21) in general show greater relationship to other members of the 21 group than they do to the 7 and 14 groups. All members of the 21 group excepting CO_4 are least related to $T.$ $monococcum$ (7).

It appears that the members of the 14 group are a homogeneous assemblage characterized by essentially the same globulins, and further that these globulins are similar to those of $T.$ $monococcum$ (7). This might be interpreted to indicate that the 14 group arose from the 7 group. That they are not identical might indicate the validity of GAINES' (21) contention that the wheats of the 14 chromosome group contain two sets of chromosomes, A and B, derived from einkorns with sets A or B. The members of the 21 chromosome group do not form so homogeneous an assemblage, greater differences existing in their globulins than exist among the globulins of the 14 group. Some of these are more like the globulins of the 7 and 14 groups than they are like those of other members of the 21 group. The globulins of all of the members of the 21 group save those of the highly resistant CO_4 are least like those of the one member of the 7 chromosome group.

Table III seems to indicate that serologically the resistant forms are closely related, and that in general they are more closely related

to one another and to the slightly resistant than to the susceptible forms. In general this applies also to CO_2, which is susceptible in the seedling stages but resistant in maturity. Practically ideal rankings are obtained when the resistant forms and the susceptible forms were tested against the others, the susceptible forms showing least relationship to the very resistant forms. When the slightly resistant form and CO_2 (which is very resistant when mature but susceptible in the seedling stage) were tested, it was found that a progressive ranking on the basis of either increasing resistance or increasing susceptibility cannot be made; in other words, their globulins are related to those of both resistant and susceptible forms, but in no regular way that could be detected by this method with the material at hand. This might be interpreted to mean that the globulins of the susceptible forms have come from forms other than the 7 or 14 forms tested. If *Aegilops* and a susceptible *T. dicoccum* had been available, a decisive answer might have been possible. The intermediate position of CO_2 is significant, indicating that it possesses globulins of the resistant and susceptible forms.

It would appear from this that the resistant forms are characterized by closely related globulins; that the susceptible forms are characterized by closely related globulins; and finally, that the slightly and varyingly resistant forms are characterized by globulins which are closely related to globulins of both resistant and susceptible forms. Furthermore, all the forms have some globulin in common, and each form is characterized by its specific globulin.

A comparison of tables III and IV seems to indicate that resistance and susceptibility of the forms studied are paralleled more closely by the presence of certain globulins than by chromosome number. Hope CO_4 (21), the resistant hybrid of an emmer (14) and Marquis (21) a slightly resistant form, shows greater relationship to members of the resistant 7 and 14 groups than it does to Marquis (21), its only moderately resistant parent. Marquis used as antigen shows about equal relationship to CO_2 (21), which is resistant when mature but susceptible when young, and to little club (21), which is susceptible, and to its resistant progeny CO_4 (21).

According to AAMODT (1), Hope is a hybrid produced by McFAD-DEN by crossing vernal emmer and Marquis, a common wheat.

Hope is known to contain the chromosome number of the vulgare group, and is also the most resistant common wheat known, showing a resistance similar to that of its emmer (14) parent.

According to LEIGHTY (10), a high degree of association has been observed between emmer characters and rust resistance in segregation of crosses between emmer and common wheat, and likewise between durum and common wheats. Numerous crosses of these kinds have been made to secure rust-resistant wheats. From the thousands of segregates that have been grown, few have been obtained combining the good qualities of the vulgare group and the rust resistance of the emmer-durum group. Evidently Hope contains the chromosome number of common wheats and enough globulins peculiar to the emmer group to make it closely identifiable serologically with the 14-chromosome group. According to VAVILOV (27), the 21-chromosome group exhibits exceptional polymorphism, representing physiologically and morphologically a very heterogeneous group of forms.

The results seem to indicate that for the material used in these tests the serological technique will differentiate and group resistant and susceptible varieties, but that apparently it detects association between serological relationship and chromosome groups only in so far as chromosome number and resistance harmonize. VAVILOV (26) states that hybrids show that a strongly marked physiological immunity to rust is a dominant character, whereas in those forms showing little immunity, susceptibility is a dominant and immunity a recessive character; and that experiments have shown that immunity is not connected with distinct morphological characters but depends upon internal physiological peculiarities of the plant. If we substitute "serologically effective globulins" for the phrase "physiological peculiarities," we have an adequate statement of the serological behavior of Hope; and furthermore a support for the idea that the serological discrepancies of the partially resistant-susceptible members of the 21 chromosome group (which are neither completely rust-resistant nor wholly rust-susceptible) are associated with or due to the very nature of their mixed globulin constitution. Accumulation of chromosomes apparently does not increase resistance, although some resistant forms have a high chromosome count, namely, Hope (CO_4).

Conclusions

This research seems to indicate that globulins can be used as an index of resistance and susceptibility phenomena in the wheats studied, and that a closer parallelism exists between ranking on the basis of globulin relations and rust resistance, than on the basis of chromosome numbers and globulin relations. In view of the fact that resistance and susceptibility characters are genetically determined, their ultimate basis must be sought in the chromosome, specifically in the genes according to present views of geneticists. The questions then arise: Did the serological technique employed reach into the chromosomes to the genes? Are globulins of the chromosomes or of the genes involved in the resistance and susceptibility phenomena of the wheats studied? Positive answers are not inescapable. It is questionable whether the proteins studied specifically enter into the nuclear make-up to any extent (WELLS 28). They probably are resident in the plasma.

All that the data seem to indicate, in the light of genetic experience with the forms tested, is that there is something (factor, gene) in the chromosomes of the resistant forms that determines the development of globulins which are in some way associated with or determine resistance, or that the factors which determine the formation of these globulins also determine resistance, or that the factors determining the formation of the globulins and the factors determining resistance are linked. These data also seem to indicate that some of the factors determining globulin formation are essentially the same, or come to expression in essentially the same way, in the resistant forms of the 7 and 14 chromosome groups. Consequently, the resistant forms of the 7 and 14 groups studied are closely related, and the 14 chromosome group may have arisen from the 7 group by reduplication; or by a cross of two einkorns with closely related but not identical chromosomes; or the 7 chromosome group may have arisen from the 14 chromosome group by loss of 7 chromosomes.

In the resistant forms of the 21 chromosome group some factors apparently are the same, or come to expression in the same manner as in the resistant 7 and 14 chromosome forms, and consequently may have been obtained from them by crossing, or in a mutation involving duplication. In the susceptible forms, however, this factor

or factors either are less active, or their action is balanced in some fashion so that a factor (or factors) for entirely different globulins come to expression. These globulins either are associated with or determine susceptibility, or their formation is determined by factors which also determine susceptibility, or their factors are linked with those determining susceptibility. Either these globulins are related closely enough to those associated with resistance to give serological group reactions with them, or the susceptible and resistant forms contain globulins of both kinds so that group reactions are obtained. At any rate the globulins associated with or determining susceptibility are of the same general nature as those associated with or determining resistance; and the factor determining formation of the former may have arisen by mutation from those determining the latter, or they may be of independent origin and therefore brought into the 21 chromosome wheats either by reduplication or by hybridization.

In the intermediate forms of the 21 chromosome group, both situations just stated for the resistant and susceptible forms exist simultaneously to a marked extent, but in varying degrees, so that globulins which are associated with or determine resistance, and globulins which determine or are associated with susceptibility occur simultaneously, or in succession in varying proportions in the same forms. It is unfortunate that a susceptible 14 chromosome wheat and a species of *Aegilops* which is susceptible were not available for this study as planned. In this manner some light might have been thrown on the nature of the factor determining the globulins which characterize the susceptible forms. Furthermore, some answer might have been reached as to whether the 21 group arose from the 7 and 14 groups by doubling and trebling of the chromosomes, or by hybridization with another form, such as *Aegilops*, which introduced a chromosome group carrying factors for susceptibility.

If the constitution of the globulins determines resistance or susceptibility, the relationship probably is not as direct or specific as that demonstrated recently by LINK, ANGELL, and WALKER (11) for protocatechuic acid and the resistance and susceptibility of the onion to *Colletotrichum circinans*.

This research seems to indicate, therefore, that serological technique may become an aid in systemtic and genetic studies of wheats.

In the forms studied it seems to be better adapted for differentiation and grouping on the basis of resistance-susceptibility characters, which are invisible characters that seem to have their basis in the constitution of globulin, or globulin complexes, than on the basis of visible characters such as chromosome numbers. One might be inclined to state that it is concerned more with so-called physiological than with so-called morphological characters, if it were not recognized that every morphological character also has its chemical basis.

This research gives promise that by the combined use of refined methods in separating the diverse protein constituents of wheat, and of more refined serological methods such as the complement fixation and the anaphylactic tests, as well as wise selection of forms of wheat and of *Aegilops*, a clue may be obtained as to the relationship of these wheats, and the source and distribution of the factors determining resistance and susceptibility.

Summary

1. Globulins of wheats of the einkorn, emmer, and vulgare groups were secured by electrodialysis in pure form and without denaturing, preliminary to use as immunizing and test antigens.

2. Relatively long periods of immunization (eight injections) of rabbits were necessary to obtain good titres in the precipitin ring test.

3. The reactions were highly specific in tests of homologous antigens and antiserums. Group reactions were obtained with the heterologous antiserums and antigens of all the wheats tested.

4. The mass precipitin test was not found satisfactory and usable.

5. The precipitin ring test gave precise and readily readable reactions.

6. A pH of 7 or one lying between pH 7 and 8.5 was necessary for the formation of rings.

7. Serological relationship seems to parallel chromosome number in the members of the 14 chromosome group studied. Parallelism between chromosome number and serological relationship does not seem as definite for the forms of the 21 chromosome group studied; some forms standing closer to forms of the 14 chromosome group than to other forms of the 21 chromosome group.

8. Serological relationship of the various forms of wheat tested

seems to be closely paralleled by relationship as measured by their resistance-susceptibility behavior to *Puccinia rubigo-vera triticina*.

9. The relationships determined immunologically by use of globulins as immunizing and test antigens seem to be more in harmony with relationships determined by breeding and inoculation, using resistance to *Puccinia rubigo-vera triticina* as the criterion, than with relationships determined cytologically, using chromosome number as the criterion.

10. The precipitin test and more refined immunological tests, using pure proteins as antigens, give promise of furnishing a clue to the relationship of wheats, their derivation, and the source and distribution of the factors that determine resistance and susceptibility.

I wish to express my thanks to Professor GEORGE K. K. LINK, of the Botany Department of the University of Chicago, who suggested the problem, for continued interest and valuable criticism during the research, and to Dr. E. B. MAINS for material and helpful suggestions relative to selection of the material used in this research.

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ECOLOGICAL STUDIES OF THE VEGETATION OF THE GREAT SMOKY MOUNTAINS OF NORTH CAROLINA AND TENNESSEE

I. SOIL REACTION AND PLANT DISTRIBUTION

STANLEY A. CAIN

(WITH SIX FIGURES)

Introduction

The Great Smoky Mountains National Park, in the North Carolina-Tennessee region of the Southern Appalachians, offers an excellent field for the investigation of soil reaction in relation to plant associations. It is in these mountains of high rainfall and luxuriant vegetation that the greatest height east of the Rocky Mountains is reached. On these lofty peaks, many of them rising over a mile in altitude above their immediate base, is found a forest rich in numbers, in species, and with a magnificent development of individuals.

Procedure

In most instances soil samples were taken in pairs, one at the surface and one immediately beneath, at a depth of 6 inches. A surface sample consists of 200 gm. or more of soil taken immediately beneath the duff. This stratum of the soil is considered important as the level of germination of seeds. The sample from a depth of 6 inches represents in most instances what may be called subsoil. In the heath bald and spruce-fir associations this depth was frequently insufficient to pass below the peaty layer, although samples from the subsoil were often equally acid. It is assumed that the 6-inch depth represents the medium of establishment of seedlings, while it is known that for many species this is about the level of greatest root development. In the pine heath the soil was frequently of little greater depth than 6 inches. In the peaty soils of birch woods, heath bald, and spruce-fir woods, woody plants were found to be very shallow rooted, while herbaceous plants were strikingly rare. What-

ever the relation of the reaction of the soil to the vegetation may be, such samples are probably adequate for pH studies of plant communities.

All determinations of the hydrogen-ion concentration of soil samples were made electrometrically on the Youden hydrogen-ion concentration apparatus. This portable apparatus was put on the market early in 1928, and was used in the field by the writer in preliminary investigations that spring. Subsequent tests, in 1928 and 1929, were made in the laboratory on soil samples which were brought in, air-dried, and tested at the earliest convenience. It has been shown by GUSTAFSON (8) and others that soils so treated do not show appreciable change over considerable periods of time. (Although the Youden apparatus is convenient for field work, the ability to collect soil samples and read them several days or even weeks later in the laboratory is more convenient, eliminating the carrying of equipment.) From ten to sixty samples were taken in each of the associations studied, selected from different locations in the same stand and from stands as many as 40–50 miles apart. Altogether 289 soil samples were determined for hydrogen-ion concentration. Three separate tests (separate soil solutions) were made on each soil sample collected, to determine the extent of very local variations.

It was found that the average deviation of three tests each for 220 samples was 0.21, or approximately 0.1 pH from the mean of the three tests. When the samples were considered in regard to their degree of acidity, it was found that the deviation averaged about the same anywhere between pH 3.5 and neutral. For example, the average of 11 samples, lying between 3.5 and 3.9 was 0.2 deviation; for 54 samples, lying between 5 and 5.4 was 0.18 deviation; and for 10 samples, between 6 and 6.4 was 0.21 deviation. On a basis of this work, some by CAIN and FRIESNER (1), and some unpublished data, totaling about 2000 tests, it is concluded that one test per sample is sufficient. In the first place, different soil samples may show a greater divergence; while such minor variations cannot be interpreted in relation to plant distribution.

The determination of acidity by the Youden apparatus permits a ready translation of the e.m.f. into pH, the common form of expres-

sion. In certain earlier work the writer, together with other investigators, has made the mistake of overlooking the logarithmic nature of the pH numbers in performing such operations as arithmetical averaging of pH determinations. WHERRY (20) has presented a valuable discussion of common mistakes in manipulation of pH data, and, primarily for the layman, a more readily understandable method of stating acidity and alkalinity. He proposes the term "active acidity" as the amount of hydrion present in the solution free to exert the effects commonly classed as acidity. WHERRY explains the concept as follows:

The amount of hydrion present in a liter of pure water is taken as the unit of acidity; it amounts to 0.0000001 gram. By use of a table of logarithms, the quantity of these units represented by each successive pH number is calculated. From the total amount of hydrion thus calculated is subtracted the amount of hydroxylion which is also present, the one being the reciprocal of the other, and the result is rounded off to the nearest 0.5.

In the study of the acidity of soils of various associations in the Smoky Mountains, the results have been expressed in range in pH, and also in "active acidity" in which the logarithmic relations are more apparent. Averages are expressed in pH because of the greater familiarity of the term. These pH numbers were obtained by changing each pH determination into its equivalent "active acidity," averaging the results arithmetically and changing the average back into pH. The importance of this step is apparent when we consider the results of incorrectly averaging pH numbers arithmetically and by active acidities, when it is found that the discrepancy is frequently as much as 15 to 20 per cent.

Preliminary results

With the work of SALISBURY (17) on the relation of pH to topography in mind, it was decided to investigate that aspect of soil reaction and vegetation in the Smoky Mountains. This seemed a promising field because of the characteristic associations, here called heath balds, which are common on the ridge tops and upper slopes at the higher elevations in the mountains. Since this association is dominated by members of the Ericales, which are notoriously acid-tolerant, and since the lower slopes and coves are occupied by com-

mon woodland types, it was thought that the pH relations to the vegetation and topography might be discerned. A line was selected which would pass from Brushy Mt. to Ball Mt., cutting across two sharp ridges between. The tops and the upper southwest slopes of these four prominences are occupied by heath bald associations. High on the north exposures and lower on the southern, the balds give way to chestnut-oak woods, and lower in the valleys to the cove type of forest (FROTHINGHAM 6). Along this line, soil samples were selected at intervals, the acidities of which are indicated in fig. 1. It will be seen that on these four prominences, and on two
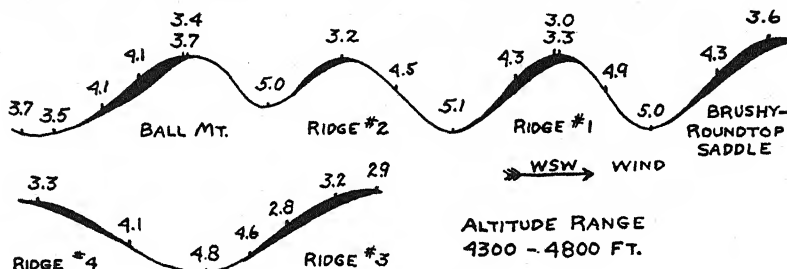
FIG. 1.—Two lines of soil samples taken across ridges to show position of heath bald associations and altitudinal relations of soil acidity (expressed in pH numbers at point where samples were taken); vertical scale is grossly exaggerated.

others near by, there is a rather characteristic distribution of the heath balds, as indicated by the broad black portions of the line. It is also apparent that, in so far as these few readings are indicative, the ridge tops are highly acid, the coves moderately acid, and the middle slopes intermediate in acidity as well as in position. Although the heath balds are generally higher in acidity than the woodland associations, it will be seen that the reaction is not related to vegetation alone, but in part at least to topography. Similar relations have been worked out for hills 100–200 feet high by CAIN and FRIESNER (1). With the stimulus derived from these preliminary results the investigation was carried on later in 1928 and 1929.

Associations

The soil samples determined for acidity have been arranged for the purpose of this study by major associations, which in turn have

been considered from the point of view of their altitudinal distribution. The following is a brief description of the associations studied.

JUNIPER WOODLAND.—Soil samples for the juniper woodland study were taken a few miles south of Knoxville, Tennessee. These soils are usually shallow, rocky, and strongly alkaline. The vegetation of this association has recently been described by PICKLESIMER (13) as the so-called cedar glades, near Nashville, Tennessee. *Juniperus virginiana* is dominant, and *Ulmus alata* and *Celtis mississippiensis* are the most important associates. *Symphoricarpus orbiculatus* and *Adelia ligustrina* are common shrubs, while *Sedum pulchellum*, *Arenaria patula*, *Erigeron ramosus*, and *Geum canadense* are common herbs. In the comparison of soil reactions with altitude, the juniper woodland cannot be considered with the rest of the associations since it is the only one with limestone bedrock.

FALLOW FIELD.—In the vicinity of Gatlinburg, Tennessee, at the foot of Mt. LeConte, which is the locus of most of this investigation, a fallow field was selected to represent the soils at the low altitude of approximately 1200 feet. This field has not been modified by application of fertilizers. The disturbance of the soil by cultivation in previous years has resulted in a more homogeneous profile, and possibly a slightly less acid condition than would otherwise have resulted.

CHESTNUT WOODS.—The soil reaction of two different stands of chestnut type were investigated from samples taken in the vicinity of 3000 feet altitude. *Castanea dentata* predominates, with a varying admixture of *Quercus rubra*, *Q. prinus*, *Fagus grandifolia*, *Acer saccharum*, *Halesia carolina*, *Carya cordiformis*, *C. laciniosa*, *Cercis canadensis*, *Robinia hispida*, *Sassafras variifolium*, etc. Elimination of the chestnut by the ravages of blight is converting this into the chestnut-oak type (FROTHINGHAM 6). The chestnut woods is a common type occurring frequently on dry north-facing slopes, and south-facing slopes to an altitude of almost 5000 feet in places. The soils are sandy and frequently shallow, and toward the higher altitudes may be semipeaty at the surface, and from subacid to low mediacid in reaction. Frequently on lower ridges the south-facing slopes are occupied by pine woods, while the north slopes at the same altitude are covered with chestnut.

PINE HEATH.—The "pitch pine-mountain pine type" of the forest-ers is one of the least valuable forest types, but is very interesting. Its most abundant species in the region of these studies are *Pinus echinata* and *P. pungens*, which occur in pure or mixed stands. Other associates of these are *P. rigida, P. virginiana, Robinia pseudo-acacia, R. hispida, Acer rubrum, Nyssa sylvatica*, and *Castanea dentata*. The shrubs and ground cover are particularly interesting, with members of the Ericaceae most striking: *Kalmia latifolia, Pieris floribunda, Vaccinium* spp., *Gaylussacia ursina, G. resinosa, Gaultheria procumbens, Epigaea repens*, etc. Among the herbs are *Pteridium aquilinum, Aletris farinosa*, and the orchids *Pogonia divaricata, Calopogon pulchellus*, and *Habenaria ciliaris*. The Compositae of the late summer season have strong southern affinities (KEARNEY 9), but as a whole this community is striking in its close resemblance to the pine-barren flora of the Atlantic coastal region.

On Tower Hill, Piney Mt., and similar locations the soft woods occupy the southern exposures, and the line of demarcation between them and the previously mentioned chestnut woods is frequently as sharp along the crest of the ridges as if the two associations were planted. Complete transition may occur within the space of two or three meters.

COVE FOREST.—The moist slopes and cove forests are divided into a number of types, according to the species or groups of species occurring as dominants. The present soil samples were taken from the valley of the Roaring Fork, on Mt. LeConte, at about 4000 feet elevation, where the following species are characteristic: *Tsuga canadensis, Betula lutea, Aesculus octandra, Halesia carolina, Liriodendron tulipifera, Fagus grandifolia, Acer saccharum, Quercus velutina, Prunus serotina*, etc. Undershrubs include an abundance of *Rhododendron maximum, Leucothoe catesbaei, Ilex opaca*, etc. Toward the upper parts of the coves, at elevations of about 5000 feet, the northern hardwoods are frequently found in pure stands of one, or at the most two or three species. The soils are loams, usually of considerable depth except on the steeper slopes, and are in the main low mediacid, although sometimes they reach low superacid reactions.

BIRCH WOODS.—Practically pure stands of *Fagus grandifolia* or of *Betula lutea* and *Aesculus octandra* are commonly found at altitudes

of 4500–5500 feet, particularly in saddles and upper cove heads, but *Betula lenta* infrequently constitutes one of these consociations. The outstanding peculiarity of the *B. lenta* forest here is the formation of raw peat at the surface of the soil. In so far as the writer knows, this is the only deciduous forest in the Great Smoky Mountains with raw peat formation. Here the formation of peat may be attributed in some way to the extreme abundance of evergreen undershrubs, of which *Rhododendron maximum* and *Leucothoe catesbaei* are commonest.

BEECH ORCHARD.—In the Great Smoky Mountains the subalpine forest is predominantly spruce-fir, but certain types of northern hardwoods can be classified as subalpine when the trees show distinct dwarfing and distortion from the influence of the factors concomitant with altitude. Such woodlands are common in certain portions of the southern Appalachians, notably in the Craggy Mountains, Black Mountains (DAVIS 3), and in the Smoky Mountains. In so far as the writer has been able to learn, this type of "orchard" woodland is not found elsewhere. The name is of local origin, in that the natives frequently refer to "beech orchards" or "chestnut orchards" when speaking of woodlands of stunted form at high altitudes (figs. 2, 3). Excellent examples of this form are to be found in the southern part of the Great Smoky Mountains, on Thunderhead Mt. and peaks and ridges southward to the Little Tennessee River, along the North Carolina-Tennessee state line. These mountains are topped by grassy balds (subalpine meadows). Toward the upper tree limits, about 4500–5000 feet, according to the exposure the trees become dwarfed, distorted, and wider spaced. In extreme cases the boles are short and stocky, with spherical crowns, attaining a total height of 10–20 feet. Reproduction in this type is predominantly, if not exclusively, by sprouting.

Farther north in the Smoky Mountains, starting with Clingman's Dome Mt., the peaks rise 1000–1500 feet higher than in the southern part of the range. Here the upper slopes are covered with magnificent forests of virgin spruce-fir. In the gaps between peaks and on the upper slopes of coves, beneath the spruce-fir, the "orchards" of northern hardwoods recur at about the same altitude as in the southern region of the grassy balds, although they do not show as striking form changes.

Soil samples for the present study were taken from beech consociations related to both the grassy balds and the spruce-fir, some 40–50 miles apart. The reactions show a striking similarity through-
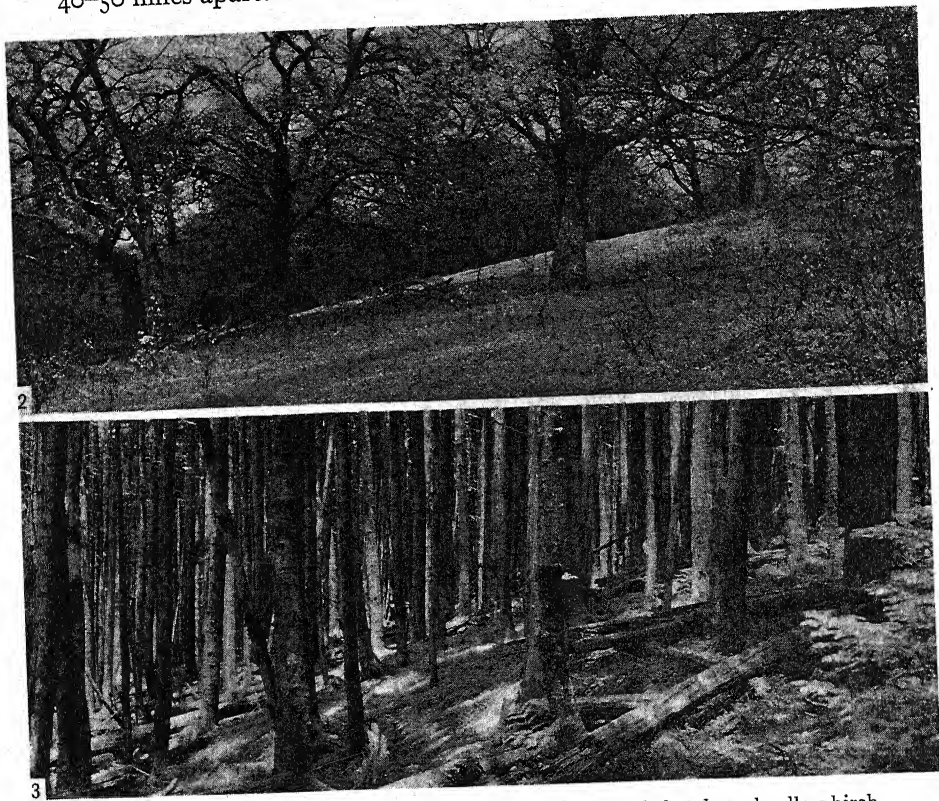
FIGS. 2, 3*.—Fig. 2, orchard woodland of chestnut, red oak, beech, and yellow birch, a subalpine type found near the margins of grassy balds; fig. 3, pole stand composed mainly of *Abies fraseri*, on Mt. LeConte at about 6500 feet elevation.

* All photographs used in this paper are copyrighted by J. Thompson Co., Knoxville, Tenn., and used through their courtesy.

out both these stands. There is absolutely no peat formation, and a complete absence of Ericaceae, although they are present in contiguous associations.

The northern hardwoods (and the beech will serve to illustrate as well as any) found scattered in the lower coves are magnificent specimens, quite equaling if not surpassing anything found in the north where they are intraneous. The pure stands of beech in the subal-

pine, however, are frequently so changed in aspect that they are hardly recognizable as the same species.

CHESTNUT ORCHARD.—The orchard type has been sufficiently considered in the preceding section. The members of the chestnut orchard are almost exclusively *Castanea dentata*, *Quercus prinus*, *Q. rubra*, and *Aesculus octandra*. The orchard woodland is formed from the cove forest by a gradual transition from the similar but floristically richer associations of lower altitudes.
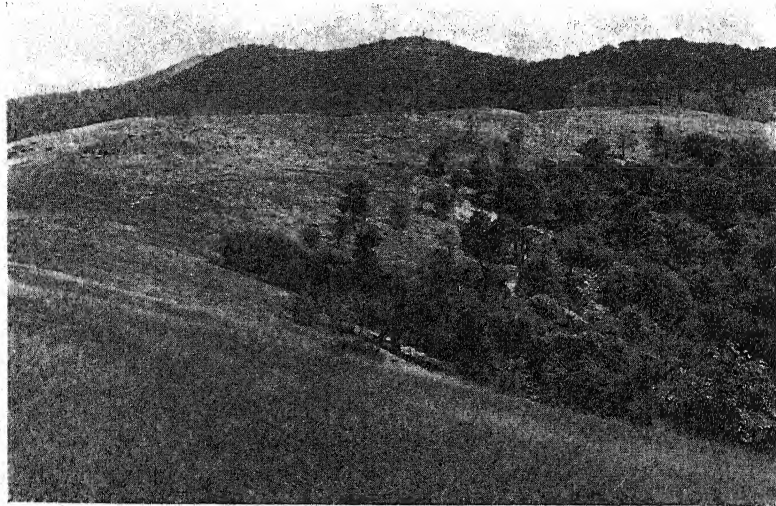
FIG. 4.—Grassy bald at about 5000 feet elevation, in southwest part of Great Smoky Mts.

GRASSY BALDS.—These treeless areas (balds) on the peaks and higher ridges of the southern Smokies are conspicuous features of the landscape (fig. 4). As has been stated, the trees begin to show striking form changes at about 4500 feet, and are completely eliminated 100–200 feet below the top, forming the mountain pastures or "alps." A number of native and introduced grasses, abundant ruderals, and a few low shrubs compose the flora. The influence of over a hundred years of intermittent grazing has made its impression on the vegetation; but in all probability these balds are natural phenomena. (This problem will be dealt with in a subsequent paper.) It is sufficient to mention that soil profiles show from a few inches

to a foot or more of homogeneous black soil of grassland type, which is too deep and mature to have developed since the advent of the white man, with the possibility of his having cleared off the trees. The eccentric growth rings of trees situated at the margin of the balds indicate that many of them have been so situated for nearly a century, although their stature is about 12–15 feet. Even had the early settlers cleared these blads, that fact would be an indication of the previous existence of open savannah woodlands with native potentiality for grassland, with slight climatic change.

SPRUCE-FIR FOREST.—This formation constitutes the majority of the subalpine forest and, as would be expected, is definitely northern in its floristic affinities. About 60 per cent of its woody flora is extraneous, the species finding the bulk of their ranges in northeastern United States, southeastern Canada, while some of them are transcontinental in high latitudes. In the last two categories are *Picea rubra* and *P. mariana*,[1] while the third dominant of this coniferous forest is an endemic, *Abies fraseri*. Endemics of narrow range and circumscribed habitat, in the southern Appalachians, constitute 27 per cent of the woody flora, including some beautiful ericaceous shrubs, as *Rhododendron catawbiense*, *R. punctatum*, *Dendrium prostratum*, and *Hugeria erythrocarpa*.

Of the conifers, Fraser's fir particularly shows a tendency to stagnate in pole stands at higher altitudes. The soil shows a decided podsolization, and is highly acid, being practically superacid throughout. The basin or "sag" on Mt. LeConte, at approximately 6500 feet, is a good example of this type of stagnant pole stand. The surface of the soil profile shows peat formation from an inch or so to over a foot in thickness, with a leached layer beneath. The ground cover consists of a dense carpet of ferns of the genera *Asplenium* and *Aspidium*, with *Oxalis acetosella*, *Clintonia borealis*, *Trillium undulatum*, and an almost continuous cover of lichens and mosses, among which *Sphagnum* species are particularly abundant.

HEATH BALD.—The so-called heath balds or "slicks" of the natives are treeless areas (fig. 5) which occur mainly as inclusions in the spruce-fir, but are also found in the region of the northern hard-

[1] Although *P. mariana* has been reported from the region, there is some doubt as to its presence in the Smokies.

woods. The physiognomy of these balds can best be described as broad-sclerophyll scrub formations; but the terms heath bald or ericad bald have a certain value, in that they suggest the importance of members of the Ericaceae. These areas are completely dominated by members of this family, which make up 40–50 per cent of the woody flora. Herbaceous plants are strikingly absent. The present tests were made from soil samples taken at about 5000 feet altitude, although some taken at about 6600 feet are considered. The under-

FIG. 5.—Heath bald in Rocky Spur region, Mt. LeConte, at about 5000 feet elevation.

lying rocks are sandstones, sand conglomerates, and quartzites, that have been much crushed and folded. The residual soils are thin and coarse, and run mediacid in reaction. On top of this subsoil is a peat deposit varying from a thin dry tundra-like crust to three or four feet of wet brown peat. In many places, particularly at the higher altitudes, the substratum is so damp and the atmosphere is so constantly humid that *Sphagnum* and associated mosses cover the ground, while *Usnea* and similar lichens are draped over the branches of the low shrubs. Although they are more characteristic of the highest peaks, the heath balds occur as low as 4000 feet in altitude, where the edaphic conditions are suitable.

Results and discussion

RELATION OF ACTIVE ACIDITY TO ALTITUDE.—In 1922 SALISBURY (17) advanced the opinion that "woodlands in general and probably all types of undisturbed plant communities in this country [England] are tending to become progressively more acid with consequent changes in the character of the vegetation." Furthermore, he concludes that there is more active leaching on high than on low ground, resulting in a depression of the upper limits of the zones as the summit vegetation extends with the extension of higher acidity. In this process of soils becoming more acid, the leaching out of bases, of which calcium carbonate is most important, increases the potentiality for acidity since bases become insufficient to neutralize the acids produced. WOODHEAD (21), in a recent consideration of the degeneration of vegetation types on the Pennine plateau, states:

> With a high rainfall leaching would go on and the soil, naturally poor in bases would become still more deficient, not only in lime, but in other alkaline bases. Here we have the beginning of those changes which not only retard and ultimately stop the decomposition of humus, but an acid condition accompanies the increase in organic matter.

In a recent extensive study of soil acidity in the forest, FRANK (5) has brought out the fact that sites above 700 meters (in the Black Forest near Freiburg) are generally more acid than corresponding sites at lower altitudes; that ridges, peaks, and exposed sites tend to become acid; upper slopes are more acid than lower slopes; and that in the soil profile, acidity tends to vary vertically as well as horizontally. In undisturbed soil profiles the highest acidity would be expected at the surface, becoming progressively less acid at greater depths, in direct relation to the extent of leaching out of bases. This phenomenon has been demonstrated adequately by SALISBURY (17), STICKEL (18), and CHRISTOPHERSON (2). In a *Vaccinium myrtillus-Betula pubescens* forest the latter finds that the raw humus layer is pH 4.0; the leached layer, 4.1; and the enriched layer, 4.5. Again, in a *Calluna-Cladonia* heath the three layers are respectively pH 4.3, 4.4, and 4.7.

In the present work, in 110 sets of surface and subsoil, taken as described at the beginning, the surface reactions averaged 0.2 of a pH more acid than the subsoils. The results of the investigation are

shown in table I, giving ranges and averages in both pH and active acidity equivalents, grouped according to plant associations, which in turn are arranged according to altitude.

The relations between surface and subsoil samples may be illustrated by a few examples. In the chestnut woods in one profile the surface soil had an active acidity of 40 (pH 5.4), and the subsoil an active acidity of 12.5 (pH 5.9). In the birch woods one profile was, surface 800 (pH 4.1), subsoil 250 (pH 4.6). While in the grassy balds many samples were the same, as both surface and subsoil 160 (pH

TABLE I

ACIDITY OF PLANT ASSOCIATIONS ARRANGED ACCORDING TO ALTITUDE

ASSOCIATION	ALTI-TUDE	AVERAGE REACTION				RANGE IN REACTION			
		Surface soil		Subsoil		Surface soil		Subsoil	
		pH	Active acidity	pH	Active acidity	pH	Active acidity	pH	Active acidity
Heath bald......	6600	3.2	6300	3.8	1600	....	....	....	....
Spruce-fir........	6500	3.6	2500	3.8	1600	4.3-2.9	500-12500	4.4-3.0	400-10000
Heath bald......	5000	3.5	2980	4.0	980	4.1-2.8	867-16000	4.5-3.5	315-3150
Grassy bald......	5000	4.8	169	4.9	141	6.0-4.3	9-473	5.9-4.5	13-293
Beech orchard....	4700	4.5	291	4.5	293	4.8-4.2	160-587	4.7-4.4	173-383
Chestnut orchard.	4700	4.6	226	4.6	241	5.0-4.4	108-372	4.7-4.5	187-315
Birch woods.....	4400	4.2	607	4.4	367	5.0-3.8	100-1733	4.7-4.4	200-400
Cove forest......	4000	4.2	630	4.6	250	5.1-3.5	80-3150	4.9-4.4	125-400
Pine heath.......	3400	4.9	140	5.0	114	5.6-4.1	28-800	5.8-4.5	16-315
Chestnut woods..	3000	5.3	55	5.3	51	6.8-4.5	1-321	5.9-4.8	12-161
Fallow field......	1200	5.9	13	6.0	11	6.3-5.5	5-31	6.4-5.6	4-25
Juniper woodland.	1000	7.9	8*	7.9	8*	7.7-8.1	5-12*	7.7-8.0	5-11*

* These nos. are in terms of "active alkalinity."

4.8), occasionally the surface soil was less acid, as in the beech orchard where one sample showed 315 (pH 4.5) and subsoil 500 (pH 4.3).

Since the influence of leaching is important in increasing acidities of upper horizons of soil profiles, the accumulated effect should be apparent in regions of uneven terrane, that is, hilly and mountainous country. More rapid leaching on high than on low ground would result in altitudinal depression of acidity zones. In the Sycamore Creek region, Indiana, CAIN and FRIESNER (1) have shown certain conformity between reaction and topography of hills about 100 feet high. Hydrogen-ion curves parallel topography curves of the ridges, the former ascending (more acid) with the latter (higher altitude).

CHRISTOPHERSON (2) reports on ten subalpine associations and five alpine associations in Sylene National Park, Norway, having a range from pH 3.6 to 7.1, but does not bring out the altitudinal relations. In the Great Smoky Mountains, however, it is possible to observe striking relations between soil acidity and altitudes, ranging from 1200 to 6600 feet. Referring to table I, we see an essentially progressive increase in soil acidity with increased altitude. This is illustrated graphically (fig. 6) for both subsoils and surface soils.
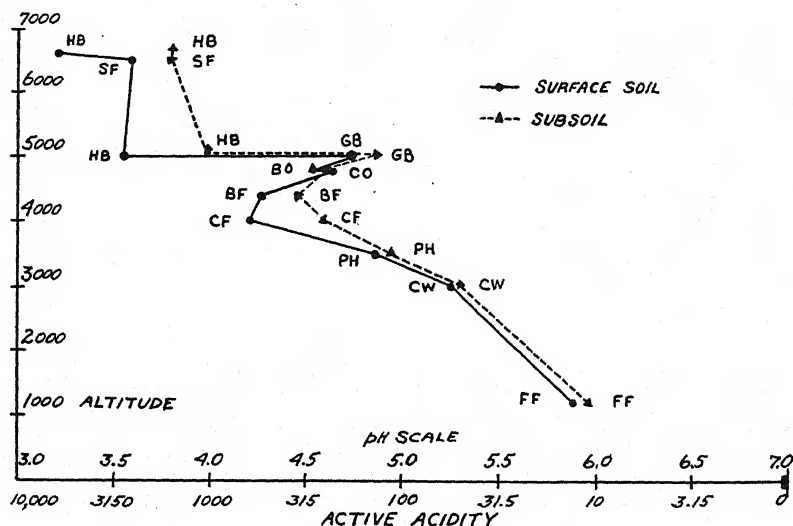
FIG. 6.—Curves constructed on basis of average reaction of plant associations plotted against altitude; symbols of associations are (from top to bottom); *HB*, heath bald; *SF*, spruce-fir forest; *GB*, grassy bald; *BO*, beech orchard; *CO*, chestnut orchard; *BF*, birch forest; *CF*, cove forest; *PH*, pine heath; *CW*, chestnut woods; *FF*, fallow field.

Assuming an altitudinal scale, that is, that the factors concomitant with increased altitude increase soil acidity, other things being equal (and they are not), those associations which are too acid for their relative altitudinal position show peat formation at the surface of the soil (as in the birch woods), or have an abundance of evergreen undershrubs belonging to the Ericaceae (as in the birch woods and the cove forest). On the other hand, those associations less acid than a curve of increasing acidity with altitude would indicate are strictly deciduous, even to their ericaceous elements (as the

grassy balds and chestnut woods, including the orchard type), or are entirely without ericads (as in the beech woods). On the whole, as indicated by the curves (figs. 1, 6), the increase in acidity of both surface and subsoils with altitude is striking, and certainly represents, among other things, the influence of long continued leaching.

In all associations but the beech and chestnut orchards the surface is more acid than the subsoil, while they are strikingly homogeneous, as indicated by the convergence of the lines in fig. 6. It will be noticed that the associations with upland peat formation (birch woods, heath balds, and the spruce-fir forest) show greater differences between the surface and the subsoil acidities, as well as greater average acidity. Of course the leaching out of bases is not the only factor in the varying acidity of the soil profiles. SALISBURY, quoting FLICHE and GRANDEAU, states:

The basic content of deciduous foliage retards the leaching process for a time, the supply of salts which the foliage contains will decrease as the soil becomes more leached till ultimately the species originally present becomes replaced by others more tolerant of acid conditions, which return an almost negligible amount of salts to the soil.

In this connection it may be observed that the associations of an entirely deciduous nature (chestnut woods and orchard, beech woods and grassy bald) are generally less acid than those with important evergreen elements.

HIGH ACIDITY PROBLEM.—The high acidity of all the mountain associations here considered requires a consideration of the factors involved, particularly since some of the associations show acidity higher than has previously been reported for plant associations in the United States. The culmination is reached in the subalpine heath balds, ericaceous communities which are here first recognized in American ecology (CAIN 22, 23).

The first factor to be considered is the nature of the underlying rocks. All the associations considered above 3000 feet are on sandstone, sandstone conglomerate, quartzite, or slate, all of which produce inorganically acid soils, usually around the mediacid portion of the scale, pH 4.4–4.6. The acidities of the various associations (leaving out the juniper woodland) may validly be compared and altitudinal differences considered, as related to leaching and addition of bases, etc.

The great age of these mountains, the oldest in North America, is an important factor in the production of high acidities of the soil, on the basis of the long duration of a number of interrelated processes. High rainfall, high humidity, and low temperature combine to provide climatic and edaphic conditions of primary importance in the development of soil acidities. There is no weather bureau anywhere in the Great Smoky Mountains, and data from the adjacent valley country, as Knoxville, Asheville, etc., are of little value. GALYON (7), however, in a study of the woody plants of east Tennessee, reports a rainfall of 83 inches for the higher slopes of the Smokies. The great humidity so characteristic of these mountains finds expression in their name, because of the almost constant fogs and clouds which rise from the damp coves, obscuring the upper slopes. One of the most apparent results of the climate is the luxuriant vegetative growth covering every available foot of surface, a rich, dense, and practically unbroken wilderness, unlike anything else in North America. This is the forest center of eastern United States and Canada, including, as it does, remnants of the ancient and once widespread Miocene flora, and the center of distribution of postglacial forests.

The climatic conditions and the luxuriant vegetation combine in the production of upland peat. The high moisture content of the soil results in low temperatures, poor aeration, and low microorganic activity, favoring poor decomposition of the vegetable litter. At the same time these factors favor the growth of *Sphagnum* and other peat-forming mosses and ericaceous shrubs and conifers capable of growing under such climatic and edaphic conditions (WOODHEAD 21). That decomposition of ericad and conifer leaves tends to produce high acidities and podsolization is well known. This has been described for pure white pine stands in New England by FISHER (4) and by STICKEL (18). That *Sphagnum* and certain other mosses tend to increase acidity of the medium in which they grow has been indicated by KURZ (10). The probability is also high that such is the case for most of the Ericaceae; that they are a factor in acidity increase, per se.

BIOLOGY OF HEATH PLANTS.—The importance of the "calcifuge" or acid-tolerant plants, in this case primarily Ericaceae, in the zones of highest acidity is apparent, and although the biology of these plants

has not been investigated by the writer, one or two of the factors involved in their success may be mentioned. It is not within the scope of this paper to discuss the establishment of the heath balds or the problems connected with the question of xeromorphy, a field reopened recently by MAXIMOV (11). It is pertinent to mention the work by THATCHER (19), however, who has shown that whenever a plant is able to produce a healthy root system in peat it is also able to obtain sufficient water from the peat to maintain a high rate of transpiration, in fact "a higher rate of transpiration than that maintained by the same plant grown under identical conditions in a loam soil." The question of "physiological drought" does not seem to exist for these plants, however; the species capable of establishing root systems in peat are few and are practically all woody and conspicuously ericaceous.

Combine with this the facts that the Ericaceae are conspicuously symbiotic with mycorhizas, and that the biology of the symbionts is intimately related to pH (RAYNER 15, 16), and we realize, as RAYNER states:

If the investigation can be carried one step farther, it should be possible to offer an explanation of the ultimate causes, not only of the calcifuge habit, but of the edaphic peculiarities of ericaceous plants in general.

MAGROU (12), who has recently reviewed the problems of symbiosis and higher plants, states that the "question of the fixation of atmospheric nitrogen by the symbionts has been reopened in connection with the mycorhizas of the Ericaceae."

Summary

1. Soils show striking variations in reaction: (a) For all soil samples tested the range was from pH 2.8 to 8.2, representing a range in "active acidity" from 16,000 to zero, and from zero to 16 in "active alkalinity." (b) The plant associations show ranges in active acidity of considerable extent, the most acid sample in an association being from about 3 to 300 times as acid as the least acid sample. In terms of pH this means a range from 4.3 to 4.8, as in the beech orchard, and from 4.6 to 6.8, as in the chestnut, based on 120 and 63 tests respectively. (c) Variations between three tests per sample

averaged about 0.1 pH from the mean of the three tests, which has increased significance with higher acidities, of course, because of the logarithmic nature of the numbers. Variations ranged from zero divergence of three tests to as much as from pH 4.5 to 5.2. (d) Surface soils tend to show greater variations than subsoils.

2. In respect to the vegetation, certain conclusions as to soil reaction may be drawn: (a) Plant associations show wide ranges of tolerance of hydrogen-ion concentration. (b) No two contiguous associations can be separated on a basis of reaction alone because of the considerable extent of overlapping both in surface and subsoils, although one association may be definitely more acid than another as a general rule (on a basis of averages). (c) Despite this situation it is apparent that the higher acidities particularly exert a considerable influence on the floristic composition of the plant associations. This is probably partly direct (influence of acidity) and partly indirect, because of the influence of peat soils and concomitant factors on the elimination of species.

3. In respect to the general high acidity, certain phenomena are conspicuous: (a) Surface soils are generally more acid than subsoils. (b) Soils of high altitudes are generally more acid than soils of lower altitudes. In a broad way this situation is apparent, but peculiar features of certain associations may enter to upset the perfect altitudinal sequence, as the evergreen habit and peat formation. The altitudinal difference is clearer when we compare identical associations, as chestnut woods at 3000 feet, with an "active acidity" of 55 and at 4700 feet with an "active acidity" of 250, or the heath bald with subsoil acidities of 980 at 5000 feet and 1600 at 6600 feet altitude.

4. Concrete evidence of one type of vegetation being replaced by a more acid-tolerant type, as a result of the tendency of soils in a humid climate to become progressively more acid, with an accompanying altitudinal depression of the limits of zones, has not been obtained as yet but is in all probability a historical fact.

NER, of Butler University, who has provided equipment and accompanied the writer in the field.

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FLORAL MORPHOLOGY OF ARCEUTHOBIUM AMERICANUM[1]

E. Silver Dowding

(WITH ELEVEN FIGURES)

Introduction

A parasitic phanerogam, *Arceuthobium americanum* Nutt., infects the soft pines in Canada and the United States. The writer first found this plant in 1928, in central Alberta, where it was growing on *Pinus banksiana* and *P. contorta.* It is a yellow plant about 4 inches high, dioecious, and leafless except for the extremely reduced bracts which are opposite and decussate, and which unite to form an inconspicuous rim at the base of each flower. Some observations were first made on the ecology and life history of *Arceuthobium*, and were published in an article on the sandhills of central Alberta (1). It was then found that the morphology of the flower itself was worthy of study, because few botanists have investigated the floral structure, and their findings are not in agreement.

Observations of North American species of *Arceuthobium* have been of a purely ecological nature; it is only on the European *A. oxycedri* (DC.) M.D. that any studies of floral morphology have been made.

In the male flower of *A. oxycedri* the perianth segments vary in number, and it is not known of how many parts the flower originally consisted. The structure of greatest interest in the male flower is the anther, which places *Arceuthobium* in a unique class among angiosperms for two reasons. First, the pollen lies in a continuous ring about a sterile column of tissue. Some observers have stated that the young anther is multilocular at first, while others hold that it is always unilocular. Second, the epidermis of the anther wall takes on the fibrous markings characteristic of the endothecium. Previous investigators seem to be in agreement about this second point. The

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female flower is known to have two perianth segments, but it is not known how many carpels there are nor how they are arranged. In short, so little is known definitely about the number and arrangement of the floral parts of *A. oxycedri* that there is only meager evidence on which to base any theory of phylogenetic relationship of the genus. This paper is intended to make some contribution along these lines.

The material used for the investigation was *Arceuthobium americanum*, parasitic upon *Pinus banksiana* and *P. contorta*. Two other species were also collected, *A. pusillum* Peck., parasitic on *Picea mariana*, and another species parasitic on *Tsuga heterophylla*, which has previously been known as *A. occidentale* Engelm., but for which ROSENDAHL (6) recommends the name *A. tsugensis*. Examination of available flowers of these two species show that their structure resembled that of *A. americanum* in all important features. For the sake of comparison with a related genus showing a better developed gynoecium, *Comandra pallida* A.DC. and *C. umbellata* (L.) Nutt. were also examined.

Male flower

PREVIOUS INVESTIGATIONS

The male flower is known to consist of a whorl of perianth segments, each bearing a sessile anther. Within the perianth is a central cushion of tissue, which some observers consider to represent the undeveloped gynoecium. Concerning other details of structure of the male flower, there is much difference of opinion.

EICHLER (2) states that the usual number of perianth segments in *A. oxycedri* is three, but that sometimes there are only two. He describes the anthers as bilocular, and opening with a common fissure. JOHNSON's (4) view of the anther agrees with that of EICHLER, and that of HOOKER whom he quotes. He notes in addition that the epidermis of the anther wall shows the fibrous markings which normally characterize the subepidermal layer. HEINRICHER (3) has frequently found tetramerous male flowers in addition to the trimerous and dimerous ones seen by EICHLER. HEINRICHER states that the anther is unilocular, and describes a central column of sterile tissue which extends to the top of the anther and is surrounded

by sporogenous tissue after the manner of the columella of a moss capsule. STAEDTLER (8), having examined the same species, agrees with HEINRICHER that the mature anther is unilocular, but states that it is originally tetrasporangiate because the archesporial tissue is at first divided into quadrants by sterile septa. PISEK (5) is not in agreement with STAEDTLER, for he finds from serial sections that the supposed septa in the anther are not complete, and the archesporium is therefore continuous. He regards the septa as projections of a tapetal nature occurring in the central "columella." He confirms JOHNSON's statement that the epidermis becomes the fibrous layer in the development of the sporangium.

PERIANTH

In *A. americanum* the perianth parts of the male flower are usually three in number, but, as in *A. oxycedri*, there are dimerous, or more frequently tetramerous, flowers. The following facts seem to show that all the male flowers of *Arceuthobium* were originally four partite. (1) A large number of male flowers of *A. tsugensis* were examined and all of them had a perianth of four members. (2) The present investigation shows that the female flower of *Arceuthobium* is tetramerous.

In the dimerous flowers examined, the perianth lobes were always opposite the subtending bracts, the lobes alternating with them apparently having failed to develop. When the flower is trimerous, the arrangement varies; the unpaired segment is usually opposite the subtending bract, but occasionally alternates with it. This variation might be explained by assuming that trimery arose from tetramery by the abortion of one or other of the members.

ANTHER

Examination of a great number of serial sections showed that the anther is unilocular, and penetrated by a sterile column of tissue in the way that the columella penetrates a moss capsule (fig. 1). This has been described by HEINRICHER. Such an arrangement is so unusual that previous workers have studied young anthers to determine whether the archesporium is always a continuous ring from its earliest stages. STAEDTLER reports that the anther is originally multilocular, but that the partitions between the locules break down at
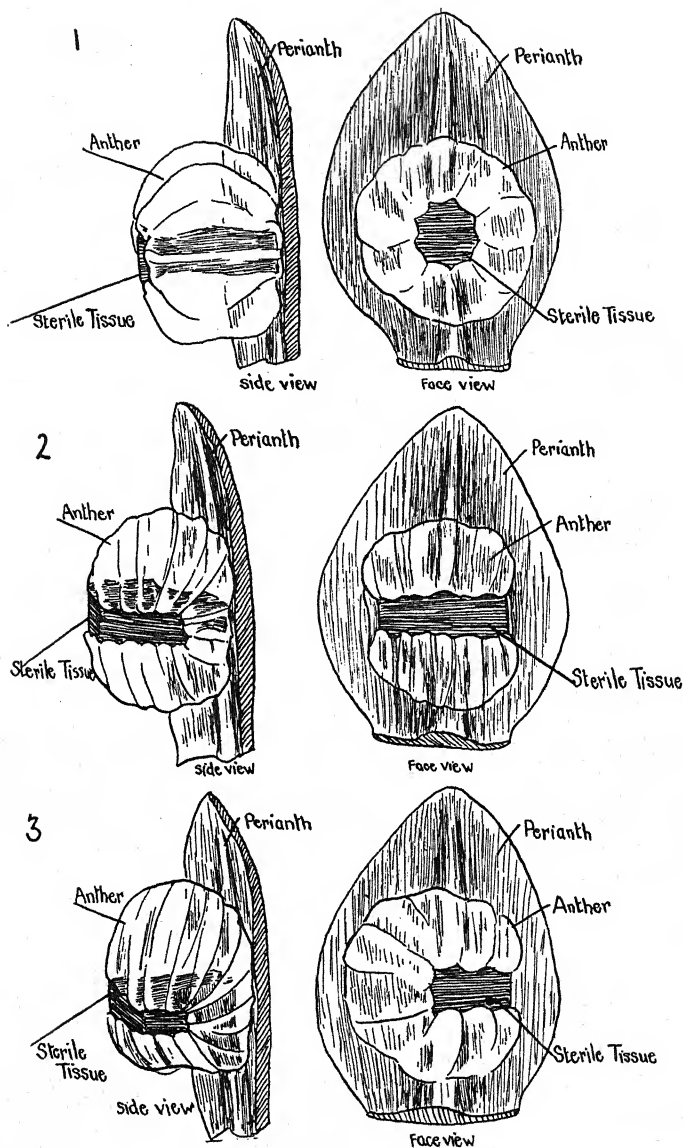
FIGS. 1–3.—Fig. 1, perianth lobes of male flowers of *A. americanum* bearing anthers; common type of anther with "columella" of sterile tissue; fig. 2, anther with sterile tissue forming partition along one plane; fig. 3, showing partitions along two planes.

maturity, leaving the "columella" in the center. PISEK states that the anther is always unilocular, and that the partitions that STAEDTLER describes are merely tapetal strands. My observations support those of PISEK, in that serial sections indicate that the archesporium is continuous in the young anther, but I am not entirely in agreement with him.

After examining a great number of anthers of all ages, I have found that the "columella" exhibits wide variations: it may take the form of a partition dividing the anther almost completely into two sacs (fig. 2); half way up it may die away and a new partition arise at right angles (fig. 3); or the partitions may give out branches which extend to the anther wall. The columella is something more than PISEK's "tapetal strands disappearing at maturity" (5). It persists in the ripe anther; and in spite of variations in position, it can always be distinguished as forming some part or other of the partitions of what apparently was once a tetrasporangial anther. These partitions, tending to divide the single archesporium into four parts, are present in every degree of incompleteness. They are four or five cells thick, only the surface layer having the character of a tapetum. From their permanence, their constancy of position, and their thickness, therefore, it may be concluded that the flanges of the columella are the surviving portions of a tissue which once separated four distinct archesporia.

The perianth of the male flower is discernible in May, and the archesporium early in June. The latter is laid down as a continuous tissue, the sterile portions never being quite complete. This confirms PISEK's observations on *A. oxycedri*, but not those of STAEDTLER on the same species, for he thought he saw four distinct groups of archesporial cells.

In examining the male flower buds in early spring, particular attention was paid to the endothecium, or fibrous layer, of the anther wall, because in the mature anther this layer is outermost instead of being hypodermal, as is the rule in angiosperms. The endothecium, the intermediate layer, and the tapetum are differentiated in early July, and the mode of development of the anther wall is identical with that of *A. oxycedri*, as described by JOHNSON and PISEK. The fibrous layer originates from the outermost layer of the anther wall.

The mature anther in the Ericaceae also has a superficial endothecium, but only because the epidermis which covers the young anther is lost at maturity. To find any families that resemble *Arceuthobium* in the possession of a truly superficial endothecium, we must turn to the Coniferae.

Reduction division of the pollen grain mother cells takes place late in July. Material collected at this time was used for counting the chromosome number. The haploid number for *A. americanum* was found to be 14. (PISEK reports 13 for *A. oxycedri*.)

Female flower

PREVIOUS INVESTIGATIONS

The female flower of *Arceuthobium* is described by taxonomists as consisting of a gynoecium and a bipartite perianth, which are fused for most of their length. The style shows a stylar canal which widens to a funnel shape in the region of the stigma (fig. 4). In the main, previous observers of the floral morphology of the female flower are in agreement.

FIG. 4.—Median longitudinal section through young female flower of *A. americanum*, showing position of embryo sacs.

EICHLER (2) represents the perianth segments as standing opposite the inclosing bract, but he does not distinguish carpels. JOHNSON (4) states that in *A. oxycedri* the ovary is made up of two carpels which, like the perianth segments, are also opposite the bract. He observed that in the cavity inclosed by the two carpels there is a central dome of tissue which terminates the axis and bears two embryo sacs in line with the two members of the perianth. HEINRICHER (3) also considers that there are two carpels opposite the perianth leaves.

GYNOECIUM

No previous observer has studied the stigma in transverse section. JOHNSON's opinion of the dual number and orthogonal arrangement of the carpels was founded on median longitudinal sections, which, when taken through a funnel-shaped stigma in any of the possible planes, naturally show a right and a left lobe. Although HEINRICHER bases his conclusions of the dual number of carpels on the shape of the stylar canal in transverse section, he gives no illustration of cross-sections of the stigma tip.

Microscopic examination of a dissected stigma showed that it was unequally bilobed (fig. 5). This led to the study of the stigma in transverse section. In the course of the present work, great numbers of transverse sections of the stigma of *A. americanum* were found to show indentations which indicated the presence of more than two carpels. Although the indentations are usually shallow, in some flowers the stigma was evidently divided into four equal parts, the segments being arranged in the diagonal planes (fig. 6). These segments were taken to be four diagonal carpels.

The four carpels fuse in varying degrees. Thus it was observed that any two neighboring carpels may be partially or completely fused (figs. 7, 9), or that the stigma may be bilobed because of three carpels fusing and one remaining free (fig. 8). The usual appearance of the stigma is that of a funnel, the rim of which is unequally bilobed (fig. 5). The larger half, as shown by incomplete fusion, is usually made up of three carpels (fig. 8). In some flowers none of the four carpels is free, and the stigma tip is an entire rim.

EICHLER has already suggested that the carpels might be alternate with the perianth segments, because in the male flowers the central cushion of tissue which probably represents the gynoecium is drawn out into corners between the perianth segments. HEINRICHER doubts that this cushion represents an abortive gynoecium, and points out that its surface shows nothing to indicate nectar secretion; but in *A. americanum* this surface bears numerous pairs of glandular cells not found elsewhere on the axis. In the tetramerous male flower this central tissue might certainly be taken to represent the four diagonal carpels.

The ovules of *Arceuthobium* are imbedded in a central papilla which terminates the floral axis, and which is surrounded by four fused carpels (fig. 4). The central papilla has been previously con-
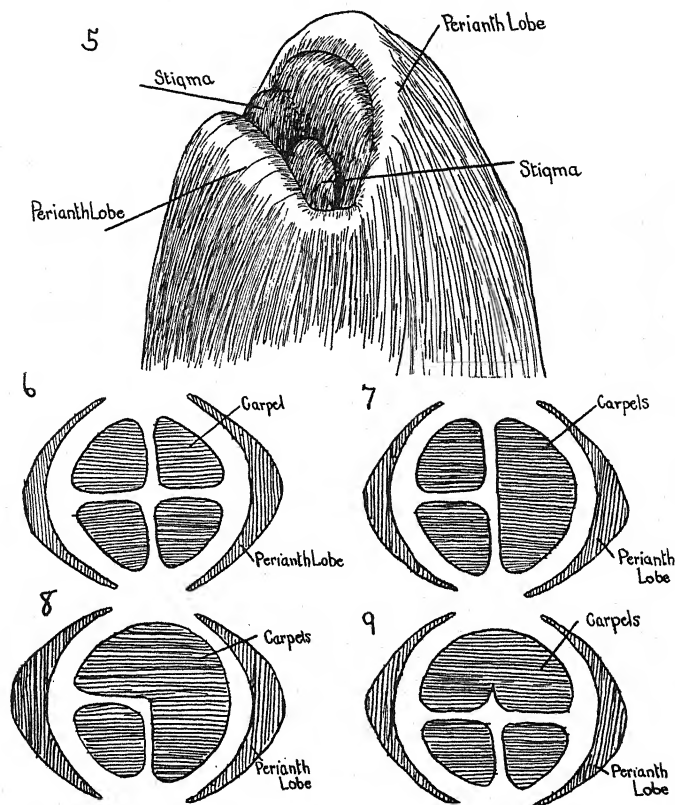
FIGS. 5–9.—Fig. 5, stigma of female flower showing unequal bilobing; fig. 6, stigma of well nourished terminal flower with four distinct carpels; figs. 7–9, stigmas showing varying degrees of fusion between the four carpels.

sidered as a placenta, that is, the bases of the four surrounding carpels are supposed to project upward to form a central column or placenta to bear the ovules. It is impossible to conceive of the outer carpels having any connection with the ovules. The ovules are situated along the plane where the fused edges of the surrounding carpels lie; that is, the ovules are alternate with the carpels. Also

in *A. tsugensis* and *A. pusillum*, the two ovules were found in the orthogonal plane and the four carpels in the diagonal planes. This arrangement leads to the following important deduction: the four outer carpels are sterile and bear only stigmas, while the embryo sacs are borne on an inner whorl of orthogonally arranged carpel rudiments.

In the middle of July the archesporial groups can be seen in the central papilla. At no time are they in the form of single cells, as JOHNSON thinks for *A. oxycedri*, for each group is made up of as many as ten cells. The number of archesporial groups in the central cone appears to be four, and not two as was previously supposed, and they are arranged so that they alternate with the sterile carpels. The gynoecium might then be represented as $G = 4 + 4$.

Although the archesporia in the lateral plane, that is, those which are to give rise to the two embryo sacs, are as a rule much more strongly developed than the two in the median plane, some evidence that the other pair of embryo sacs may sometimes develop at the expense of the lateral pair has been found in a single terminal flower, which will now be described.

Usually the inflorescence terminates in a cluster of three flowers, and these are oriented so that the planes of symmetry through the embryo sacs are parallel, and, at the same time, are all at right angles to the planes of symmetry through the embryo sacs of the two lateral flowers at the node below. When the lateral flowers of the terminal cluster do not develop, so that the terminal flower is solitary and surrounded by two empty bracts, the plane of symmetry through the embryo sacs of the terminal flower is still at right angles to that of the laterals on the node below (fig. 10). One solitary terminal flower was found, however, which had its plane of symmetry through the embryo sacs parallel to the laterals of the node below (fig. 11). This change in the plane of symmetry of the terminal flower to a plane at right angles to the usual one (cf. figs. 10 and 11) indicates that perianth segments may develop which alternate with those usually found, and also that alternate embryo sacs may develop at the expense of those ordinarily encountered.

The development of the alternate pair of embryo sacs might be expected, since it has been shown that in *A. americanum* four archesporia are laid down. A similar alternate development for the

perianth is possible because the two segments that normally appear do not occupy the whole circumference of the perianth tube (fig. 5). (In *Viscum* the perianth is four partite.)
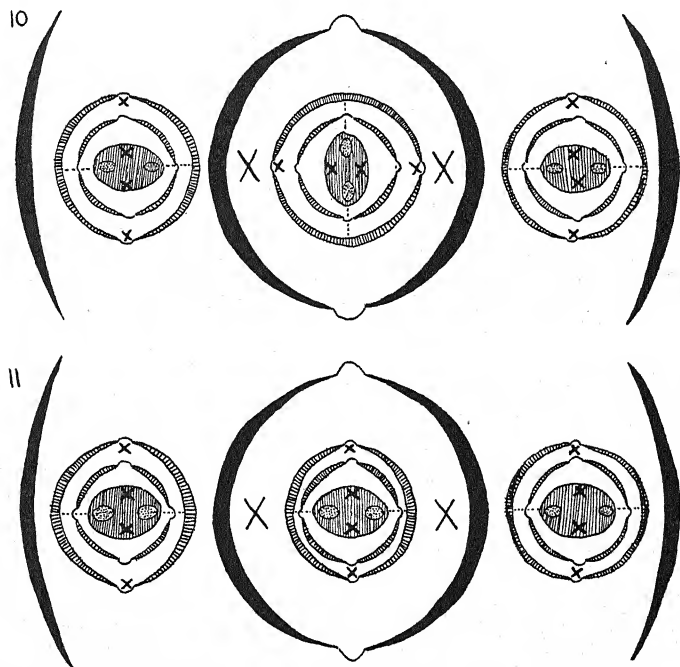
FIGS. 10, 11.—Floral diagrams of terminal clusters of female flowers (composed of three whorls, outer, the perianth and inner two, the gynoecium; bracts shown black; *x*, aborted flowers or aborted members of floral whorl); fig. 10, usual arrangement of terminal flower in relation to laterals of node below; fig. 11, unusual arrangement of terminal flower in relation to laterals of node below, indicating that alternate pair of embryo sacs has developed in terminal flower in place of usual ones. Planes of symmetry shown by dotted lines.

COMPARISON WITH GYNOECIUM OF COMANDRA

Strong evidence that there is an alternation of sterile and fertile carpels in a number of families of flowering plants has been brought forward by SAUNDERS (7). Additional evidence supporting the carpellary polymorphism of *Arceuthobium* has been sought for in the Santalaceae, because in this related family reduction due to parasitism has not gone so far.

As compared with the evascular nature of the ovary of *Arceutho-*

bium, in the gynoecium of *Comandra* the ovary wall has a well marked vascular system. The ovarian papilla of *Arceuthobium* is replaced by a spirally twisted "placenta" bearing pendulous ovules and containing a vascular strand. The number of the members making up the ovary cannot be determined by any indentation of the stigma, as this is entire, nor can any help be obtained from a determination of the position of the embryo sacs, as these are borne on a twisted column. There is only the vascular system on which to depend.

In *C. umbellata* and *C. pallida* the perianth tube is traversed by five main vascular bundles, which run to the five sepals and their superposed stamens. Five vascular strands branch off from these bundles and, taking up a position alternate with the main bundles, run for a short distance into the base of the carpels. Farther down in the peduncle five other vascular strands arise, which are alternate to the five bundles which run to the base of the carpels. After anastomosing, they pass into the central column bearing the ovules. The whorl of vascular tissue which supplies the "placenta" is alternate to the one which supplies the outer carpels. This indicates that the gynoecium is composed of two alternate whorls of carpels, rather than one whorl bearing a placenta, as previously supposed.

FORMATION OF EMBRYO SAC

The ovules of *Arceuthobium* are without integuments, and consist simply of embryo sacs imbedded in the central papilla (fig. 4). According to JOHNSON, in the development of the archesporium in the female flower of *Arceuthobium* a number of tapetal cells are cut off. Not the slightest evidence was found to support this view. The embryo sac mother cell divides to form two daughter cells, the upper of which is the embryo sac. In some flowers the lower daughter cell is much smaller and soon disintegrates. The embryo sac then develops normally. In other flowers the upper cell and lower cell are equal, and for some weeks keep pace with each other in further development, the nuclei dividing simultaneously. The embryo sac eventually enlarges at the expense of the lower cell and forms seven nuclei in the usual way.

Summary

MALE FLOWER

1. The male flower is thought to be originally tetramerous.

2. The anther, although unilocular, is found to be characterized by a system of sterile tissue which represents what is left of the separating partitions of a tetrasporangiate anther.

3. The haploid chromosome number is 14.

FEMALE FLOWER

4. The ovary wall is made up, not of two fertile but of four sterile carpels. These are diagonally arranged and show varying degrees of fusion.

5. The two embryo sacs in the central papilla lie in the orthogonal plane, and therefore must be borne on an inner alternating whorl of orthogonally arranged carpel rudiments. This inner whorl bears not two but four archesporia, only one pair forming embryo sacs. The structure of the gynoecium must therefore be represented as $G = 4 + 4$ and not as $G = 2$.

6. The archesporia are not unicellular but multicellular.

7. The gynoecium of the one genus of the Santalaceae examined resembled *Arceuthobium* in being composed of two alternating whorls of carpels.

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A GROUP OF TETRAPLOID ROSES
IN CENTRAL OREGON[1]

Eileen Whitehead Erlanson

(WITH THREE FIGURES)

In the course of a western collecting trip in 1928, the writer was taken by Dr. H. M. Gilkey to a large colony of interesting wild roses near Corvallis, Oregon. Hedgerows along either side of a lane consisted of *R. pisocarpa* A. Gray (diploid) and related forms, also *R. nutkana* Presl (hexaploid) and some related forms, and the rare species *R. durandii* Crépin, which is apparently confined to the Willamette River region (Erlanson 3). Two large bushes were also observed on one of which all the hypanthi had aborted, the other showing about 50 per cent of aborted fruit. These were judged to be hybrids between one of the hexaploid forms present and *R. pisocarpa*.

In the spring of 1929, young buds from four individuals in this colony (including *R. durandii*) were kindly fixed by Dr. Gilkey in acetic alcohol and forwarded to Michigan. Stages in reduction division were found in each collection. Herbarium vouchers also were collected by Dr. Gilkey from the bushes which provided the cytological material. All these bushes resemble *R. nutkana* in habit and general morphological characteristics, including the inflorescence of large flowers, borne either singly or two to three together on short lateral branches. This type of inflorescence I had heretofore found always associated with the hexaploid condition in American roses. *R. durandii* is unique among the wild roses on this continent in possessing puberulent branches and large compressed alate prickles, which are also puberulent. It has been regarded by several students of the genus as related to *R. nutkana* (Rydberg 12). It was a distinct surprise, therefore, to find that three of the collections sent by Dr.

GILKEY, including *R. durandii*, were tetraploids with twenty-eight somatic chromosomes and fourteen pairs at diakinesis.

One of the plants, which in the autumn of 1928 bore a large proportion of aborted fruit, was found to be a balanced hexaploid with twenty-one pairs at heterotypic metaphase in the pollen mother cells. One megaspore mother cell was found with the chromosomes all paired on the equator of the first meiotic spindle. In a second megaspore mother cell, which had been cut, all forty-two chromosomes were scattered about the cell at what should have been diakinesis
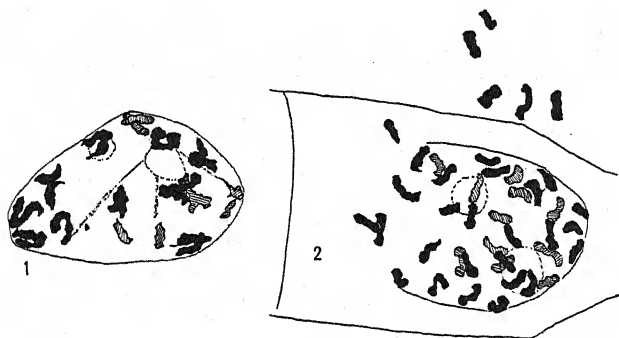
FIGS. 1, 2.—Fig. 1, diakinesis in megaspore mother cell of *R. durandii;* 13 pairs and 2 univalent chromosomes. Fig. 2, megaspore mother cell of *R. nutkana* var. from Oregon: diakinesis, no pairing, 42 univalent chromosomes; cell cut, 5 chromosomes in adjoining section shown at side of main part of cell; ×2000.

(fig. 1). These roses are distinctly protandrous, in that the megaspore mother cells are entering reduction division just before the bud is ready to open, and the anthers contain mature pollen. Since it is difficult to find many megaspore mother cells undergoing meiosis, fig. 1 is presented as an interesting observation, although no deductions can be made as to its significance in relation to the partial ovule sterility of the bush until more stages in embryo sac production have been seen. Such a nucleus might give rise to a diploid megaspore or to an apogamous embryo. Pollen taken from the herbarium specimen sent with the cytological material showed only 12 per cent of empty shriveled grains after treatment with aceto-carmine. The writer has examined pollen samples from numerous hexaploid roses, both in the group of *R. acicularis* Lindl. and of

R. nutkana, and has found less than 15 per cent of empty and dwarf grains in all plants but one, and usually less than 10 per cent.

Reduction division in the three tetraploid specimens is almost always regular, with infrequent cases of lagging of one or two pairs at first metaphase and of failure to pair on the part of two of the chromosomes. Fig. 2 shows diakinesis in a megaspore mother cell in *R. durandii*. Groups of four chromosomes in a ring are rare, and when present one group only has been observed in a cell. These are the same irregularities as those found in *R. californica* and in eastern tetraploid species (2, figs. 38, 39, 49).

Pollen taken from Dr. GILKEY's herbarium specimens showed that the *R. durandii* bush had 14.8 per cent sterile pollen. A second tetraploid had 16.6 per cent, and the third 54.6 per cent empty grains; these were not dwarf grains such as result from polyspory but full sized spores whose content had disintegrated after tetrad formation. In some anthers whole blocks of pollen grains had no protoplasmic contents. This type of pollen sterility, which is not associated with unusual meiotic irregularity, resembles that found in the sterile diploid type of *Primula kewensis* (DIGBY 1, NEWTON and PELLEW 8). In the pollen sample exhibiting 54.6 per cent sterile grains, one giant grain twice the volume of a normal grain, with protoplasmic contents, was seen. Such grains are not infrequent in rose pollen exhibiting a high sterility. They indicate the occasional failure of reduction in some spore mother cells, which have probably undergone what ROSENBERG (9, 10) designated as a "hemiheterotypic division."

TAXONOMIC POSITION OF FORMS.—The hexaploid specimen is a vigorous, much branched bush over 5 feet high. The vouchers sent by Dr. GILKEY fit the descriptions of *R. nutkana* given by RYDBERG (11), except that the branches are completely unarmed.[2]

The tetraploid specimens are *R. durandii* and two related forms, both of which possess the solitary flowers, glandular hispid pedicels, and leaflets (both glandular and puberulent beneath) of that species. They differ in having glabrous stems and prickles. One speci-

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[2] RYDBERG suggested (Bull. Torr. Bot. Club 44:69. 1917) that the unarmed specimen from the Aleutian Islands designated as *R. aleutensis* by CRÉPIN (1875) may have been an unarmed form of *R. nutkana*.

men has straight stout prickles and might be a tall form of the dwarf
R. yainacensis Greene. The plant with semisterile pollen has small
curved prickles and the branches are sparingly glandular-hispid at
the tips; it might be a stout form of *R. myriadenia* Greene which is
known only from the type locality in Jackson County, Oregon.
These tetraploid plants are not so tall nor so much branched as is
typical *R. nutkana*, and in the field recalled some of the Gallicae
(ERLANSON 3). Plants similar to them in habit were also collected
at Crescent City and Fort Bragg, California, in 1928, and were de-
termined as *R. brownii* Rydberg and *R. muriculata* Greene. The
Botanical Garden of the University of Michigan has a hexaploid
specimen of *R. muriculata* (no. 2931, Seattle, Washington, legit T. C.
Frye), with graceful branching stems 6 feet high.

We have, therefore, a group of tetraploid roses of somewhat local
distribution in central and southern Oregon, probably extending
into northern California. They resemble the hexaploid roses in the
group of *R. nutkana*, and the distinguishing morphological charac-
teristics which they possess are of doubtful taxonomic value. Prickle
shape is highly variable in nearly all rose species. All the polyploid
roses of the Pacific Coast region of the United States have stout
compressed prickles; in the group of *R. nutkana* they are usually
straight, and in the group of *R. californica* C. & S. usually curved.
Specimens of *R. nutkana* with pubescent stems have not been re-
ported. This characteristic was observed by me in an apparently
diploid specimen in Utah and in *R. ultramontana* S. Wats. in north-
western Nevada. Specimens belonging to the group of *R. californica*
from San Luis Obispo, collected by *Mrs. R. W. Summers* (nos. 256
and 258, 1903), are in the herbarium of the University of California;
they have the tips of the branches and pedicels often puberulent or
finely glandular. Other specimens related to *R. californica* have been
seen with the tips of the branches glandular hispid. This character
is therefore not restricted to *R. durandii* alone on this continent.

In order to discover whether there were any other morphological
characteristics by which these tetraploid roses could be distin-
guished, the stamens and the teeth on the leaflets were counted.
Although the serrations along each side of the leaflets are scarcely
more numerous than in the Oregon hexaploid specimen (table I),

they are distinctly finer and more like those of *R. pisocarpa* (fig. 3). The number of stamens in the four Oregon specimens is given in table I. The hexaploid has 76–85 and the three tetraploids 110–130.

TABLE I

SOME QUANTITATIVE CHARACTERS DISTINGUISHING FOUR OREGON ROSES

CHARACTERS	R. NUTKANA VAR.	R. DURANDII	R. MYRIADENIA VAR.	R. YAINACENSIS VAR.
Stamens in two flowers....	76 85	110 112	115 130	113 124
Sterile pollen............	12%	14.8%	54.6%	16.6%
Size of round pollen grains..	26.6–30 μ	25–26.6 μ	23.3–26.6 μ	....
No. of teeth on side of leaflets	9–14	10–17	10–17	9–15
Mean number of teeth......	10.2	12.9	13.2	10.7

Since the counts were made on dried specimens they are likely to err on the low side. Counts of stamens made on living plants have shown that *R. nutkana* has 90–110 and *R. californica* 105–130 stamens.

Studies on rose pollen have shown that the size of the microspores increases with the increase in the chromosome number, as in wheat (SAX 13) and other polyploid genera. In table II are listed the sizes of grains found in rose pollen examined in samples from 400 individuals of the section Cinnamomeae in the Botanical Garden

FIG. 3.—Serrations of leaflets in rose specimens from Corvallis, Oregon: (*a*) *R. nutkana* var., (*b*) *R. durandii*, (*c*) *R. myriadenia;* ×2.

of the University of Michigan. Only those grains which appeared to be perfect morphologically were measured. There is some overlapping between the sizes of the spores in the three cytological groups diploid, tetraploid, and hexaploid. The variation in pollen grain size shown for each of these groups is found in almost all samples, so that the average size for any individual in the same group will be about

equal. When rose microspores reach maturity they are shaped like grains of wheat, with rounded ends, in which condition they are usually shed from the anthers; later they become spherical. The size differences between pollen from plants with unlike chromosome numbers (table II) are more easily observed when the spores are oval in shape.

TABLE II

SIZES OF POLLEN GRAINS IN DIPLOID, TETRAPLOID, AND HEXAPLOID ROSES

CYTOLOGICAL TYPE	LENGTH OF OVAL GRAINS (μ)	DIAMETER OF ROUNDED GRAINS (μ)
Diploid...............	30–39.4	20–25
Tetraploid............	38.3–46.6	23.3–26.6
Hexaploid............	45–51.6	25–30*

* Very few samples of hexaploid pollen have been seen in which the grains have rounded off.

Three of the Corvallis specimens showed only rounded grains. The spores of *R. durandii* and of *R. myriadenia* var. were distinctly smaller than those of the *R. nutkana* specimen (table I). Oval spores were obtained from the fourth specimen which were 40–43.3 μ long, which would class them as tetraploid without much doubt. In *R. californica* (tetraploid) oval pollen grains vary from 40 to 46 μ in length.

In the light of the cytological findings in the Corvallis roses and of field and herbarium studies, I am led to suspect that the following rose species are tetraploids:

OREGON	NORTHERN CALIFORNIA
R. durandii Crépin	R. brownii Rydberg
R. myriadenia Greene	"R. muriculata" (in southern part of its range)
R. yainacensis Greene	R. calavera Greene
R. delitescens Greene	R. pinetorum Heller

Measurements are given in table III which were made on the pollen taken from three herbarium specimens not collected by the writer. The specimen collected on Mount Eddy by HELLER, and determined by him as *R. delitescens*, does not agree with GREENE's description. It is clearly a hexaploid judging from the size of the

microspores. It also has the low percentage of sterile grains characteristic of American hexaploids. It is probably a glabrous and eglandular form of *R. nutkana*. The specimen of *R. brownii* might be either tetraploid or hexaploid by the size of the rounded pollen grains. The high percentage of sterile grains suggests that it is more likely to be tetraploid.

TABLE III

POLLEN MEASUREMENTS FROM HERBARIUM MATERIAL OF WESTERN ROSES

SPECIES* AND LOCALITY	SHAPE OF GRAINS	SIZE IN μ (DIAMETER OR LENGTH)	PERCENTAGE STERILE GRAINS	INFERRED CYTOLOGICAL CLASS
R. delitescens, Mt. Eddy, Calif. Leg. A. A. Heller, no. 13254, June 20, 1919..............	Oval	45–51.6	11.6	Hexaploid
Same, no. 13507..............	Oval	45–50	....	Hexaploid
R. brownii, Trinidad, Humboldt Co., Calif., Leg. H. H. Smith, no. 3821, June 7, 1911.......	Round	23.3–26.6	37.5	Tetraploid
R. pinetorum, Pacific Grove, Calif., Leg. A. A. Heller, June 3, 1903......................	Round	23.3–25	55.6	Tetraploid
R. yainacensis, Stirling, Butte Co., Calif., Leg. A. A. Heller, no. 10809, June 7, 1913.......	Round	23.3–25	41.3	Tetraploid

* The species were determined by the writer except for the first two specimens.

POSSIBLE ORIGIN OF NEWLY FOUND TETRAPLOID GROUP

Following HURST's theory (6, 7), the discovery of tetraploid forms resembling *R. nutkana* and growing near the southern limit of its range, one might suppose that they had been derived from it by the loss of one double septet (fourteen chromosomes). Such a supposition is not in accord with the cytological behavior of polyploid species in genera which provide good material for experimental work. That the higher polyploids in *Rosa* were originally built up by the processes of hybridization and chromosome duplication is admitted by HURST (7), and it is more in line with our present knowledge to postulate that the chromosome number may be reduced by the intervention of hybridization between species having different chromosome numbers. An alternative explanation, therefore, is to suppose

that the hexaploid *R. nutkana* and the tetraploid *R. californica* have hybridized in the extreme southern region of Oregon, where their ranges overlap. Such a crossing would give an unbalanced pentaploid in F_1 with possibly fourteen pairs and seven unpaired chromosomes at reduction division. Many of the F_1 gametes would probably receive only fourteen chomosomes, because the unpaired chromosomes usually lag during the anaphases of meiosis, and an almost fertile tetraploid race might be produced strongly resembling one of the original parent species but exhibiting some characters of each parent.

Examples are known in other polyploid genera in which interspecific hybridization gives rise to offspring resembling only one of the parent species. In a cross between *Nicotiana tabacum* and *N. sylvestris*, GOODSPEED and CLAUSEN (4) obtained F_1 plants which resembled the *tabacum* parent entirely. When these were backcrossed to the *sylvestris* parent, the resulting offspring resembled *N. sylvestris* (5). Plants from a cross between *Triticum dicoccum* (x = 14) and *T. vulgare* (x = 21) yielded almost all *dicoccum*-like individuals in F_2, in the experiments of THOMPSON and HOLLINGSHEAD (14). They also found that the majority of the F_2 plants showed fourteen bivalent chromosomes and only 0–4 univalents at meiosis. These investigators attribute this to the lagging of univalent chromosomes in the F_1.

Another crossing that might be considered is one between *R. nutkana* and a diploid of the *R. pisocarpa* group, followed by autosynezesis among the fourteen unmated *R. nutkana* chromosomes at first metaphase in the F_1. One would not then expect to find such strong, variously curved prickles, such a preponderance of ·glandular foliage, such pubescent branches, nor such an increase in the number of stamens, in the descendants of such a cross. *R. pisocarpa* has fewer stamens than even *R. nutkana;* at least this is true for two plants from Oregon which have been able to persist in Michigan.

These tetraploids are unique among American tetraploid roses yet observed by the writer in not seeming to produce flowers on the annual shoots or turions, and in having a simple inflorescence of from one to three (rarely four) flowers, as in our hexaploid roses (ERLANSON 2). The ability to flower on turions is best developed

in the group of *R. arkansana*, and is only occasionally seen in *R. californica* and its relatives.

Summary

1. Cytological material from Corvallis, Oregon, of *Rosa durandii*, *R. myriadenia*, and *R. yainacensis* showed that these are balanced tetraploids with fourteen pairs at diakinesis. A semisterile *R. nutkana* was hexaploid.

2. These tetraploid roses resemble *R. nutkana* in the size of flowers and hips, and in inflorescence type. In the number of stamens, fine serrations of the leaflets, size of pollen grains, and amount of sterile pollen they resemble the tetraploid *R. californica*.

3. This tetraploid group seems to have a limited range, perhaps from central Oregon to the northern counties of California. It may have originated from a crossing between *R. nutkana* and *R. californica*.

4. It is suspected that some other rose species of limited range in this area belong to this group. They are *R. delitescens*, *R. brownii*, *R. calavera*, *R. pinetorum*, and the forms in northern California which are usually classified as *R. muriculata*.

5. It is suggested that pollen grain size might be used in distinguishing the tetraploid and hexaploid rose forms in herbaria. The cubes of the mean length of the pollen of diploid, tetraploid, and hexaploid are as 1: 1.7:1.9.

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MERISTEM IN OSMUNDA CINNAMOMEA

GEORGE L. CROSS

(WITH FIFTEEN FIGURES)

Introduction

The stele of the Osmundaceae has interested morphologists and anatomists for the past half century. DE BARY (9) first called attention to the fact that *Osmunda* in many respects exhibits a certain similarity to the type of cauline vascular system found in the majority of the seed plants. FAULL (10) described the adult anatomy of the group but did not attack the problem from the standpoint of the origin of tissues. JEFFREY (12) prepared a monograph in defense of his theory of the cortical origin of pith, using living and fossil forms of *Osmunda* to illustrate a series of advancement, beginning with *O. skidegatensis* (Jurassic) and ending with *O. claytoniana*. VAN TIEGHEM used practically the same series equally well in defense of his stelar theory. Despite the variation in interpretations of different investigators, however, it is generally conceded that the stele of *Osmunda* is very different from that of most other pteridophytes.

The present study was undertaken with the idea that a detailed investigation of the growing tips of the three prominent living species of *Osmunda* might throw light on the anatomical situation of the family as a whole. Although this paper is concerned almost entirely with *O. cinnamomea*, it is planned to make subsequent comparative studies of *O. claytoniana* and *O. regalis* before drawing conclusions relative to the phylogenetic anatomical situation of any of the species.

Material and methods

The material for the investigation was collected by the writer in the valley of the Calumet River, near Chicago.

Sections of the rhizomes were prepared for study by the paraffin method exclusively. To aid in cutting, the mass of sclerenchyma invariably found in the pith of *Osmunda cinnamomea* was dissected

out by making a longitudinal slit on one side of the stem through the cortex and stele when it was desired to cut sections any appreciable distance from the apex of the stem.

Much trouble was experienced in selecting appropriate killing and fixing reagents. Formalin-acetic-alcohol in any of the proportions recommended by CHAMBERLAIN[1] caused plasmolysis and rupture of the cells at the apices of root, stem, and leaf. Formalin-alcohol alone gave scarcely better results. Corrosive sublimate, hot or cold, occasionally gave good fixing, but as a rule failed entirely. Chromo-acetic acid penetrated very poorly and in many instances caused maceration of the material. Perhaps the most satisfactory killing and fixing reagent employed was 10 per cent neutral formalin, although some destruction of the large apical cells occurred by this method.

General anatomy

The rhizome is a massive, semisubterranean, slow growing structure, having heavy leaf bases and an outer cortex impregnated with sclerenchyma. The stem branches, probably dichotomously. The leaves arise at the apex, and in acropetal succession form a close spiral, the phyllotaxy of which has been worked out by FAULL (11). The leaf traces are concentric and endarch. The stem, to use JEFFREY's nomenclature, is a phyllosiphonic mesarch siphonostele, varying from the amphiphloic condition (*O. skidegatensis* and sometimes *O. cinnamomea*) to the ectophloic (*O. regalis* and *O. claytoniana*). Maturation at the tip of the rhizome proceeds slowly, and in consequence all tissues mature at a relatively great distance from the apex, although the xylem and protophloem are differentiated early. The roots arise opposite the wings of metaxylem, two for each leaf trace in *O. regalis* and *O. claytoniana*, and but one in *O. cinnamomea*.

STEM TIP

An apical cell or a group of horizontally seriated initials is always present. The first major work on the stem apices of the Osmundaceae was done by BOWER (2), who investigated *O. regalis* in detail. He describes the growing tip and reports finding a four-sided initial cell in a few apices. He states that there is present in most forms a

[1] Methods in plant histology. Chicago. 1915.

conical apical cell which is rather deep in proportion to its height, but he concludes that the apical meristem on the whole is of a "less regular and definite type than in the leptosporangiate ferns."

My investigations were concerned almost exclusively with *O. cinnamomea*, although a sufficient number of preparations of *O. regalis* were made to verify the work of BOWER. In general topography, the apex of *O. cinnamomea* is much the same as the apex of *O. regalis*. The cells are relatively large, thin walled, and watery.

The apices of a great number of rhizomes were sectioned. Plants of all ages and sizes were selected, from the sporeling stage up to extremely old rhizomes. Both longitudinal and transverse sections were prepared.

In the sporeling, as noted by CAMPBELL (7), a triangular pyramidal apical cell is prevailingly present. This cell persists during the youth of the rhizome, and frequently may be found in middle aged plants. The relative size and shape of the cell are shown in fig. 11. Obviously the segmentation does not proceed with the regularity characteristic of the typical leptosporangiate fern. The segments are not cut off in spiral succession as a rule, although this mode of growth sometimes occurs intermittently for a small number of segments (4–6). The identity of the segments is soon lost, due to the relatively great length of time elapsing between successive segmentations, and to the numerous divisions occurring in the segments themselves.

Before the young rhizome forks, the apical cell apparently divides into two equal portions, in each of which is organized a new apical cell that functions as the growing point of the divergence. Fig. 9 was drawn from the growing tip of a young rhizome, which was about to fork in the dichotomous manner described.

As the rhizome ages, segmentation at the apex becomes progressively less regular, and differential growth, or twisting of the stem tip due to growth of the leaves, may pull the apical cell into such position that it appears to be four-sided (fig. 14). As the segmentation becomes more and more irregular, the identity of the original triangular pyramidal apical cell becomes lost, and it is replaced by a group of initials which function as a meristem (fig. 10). The old rhizomes may branch, but infrequently. FAULL records *Osmunda* as
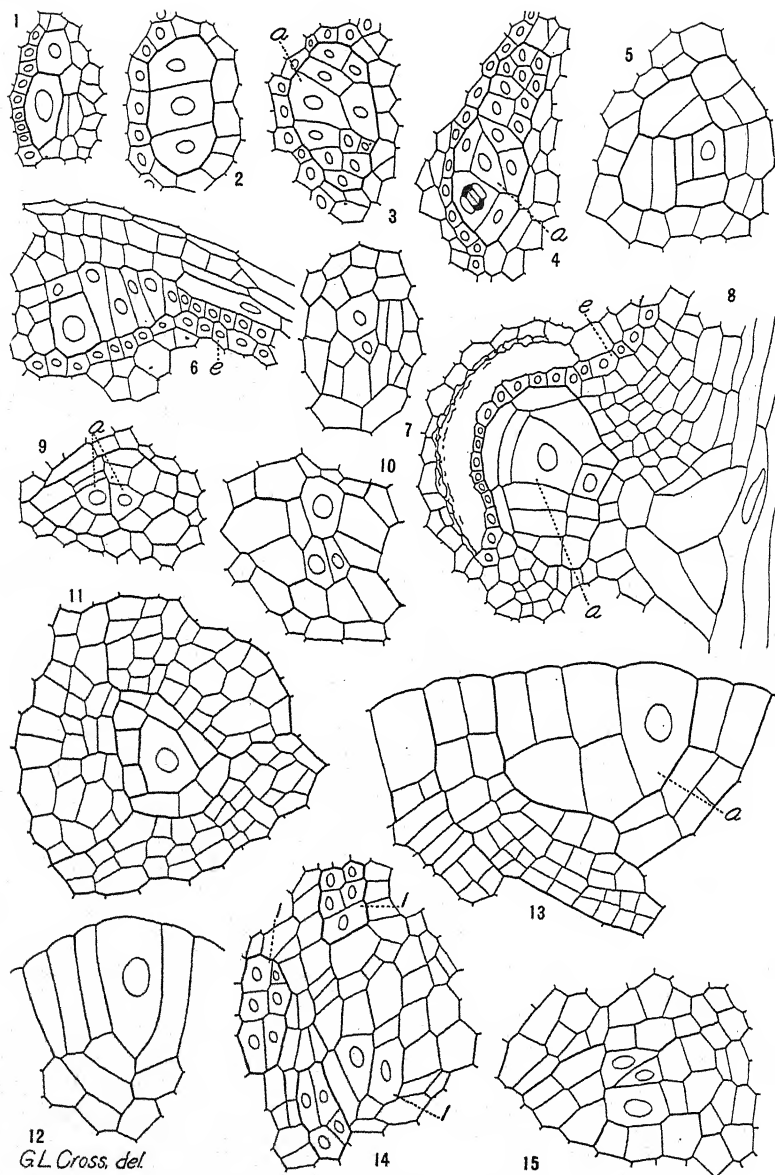
FIGS. 1–15.*—Fig. 1, young root initial arising in pericycle just within endodermis
(at left); cross-section of rhizome. Fig. 2, young root in 3-celled stage with endodermis

branching once. I have observed several cases in which a single rhizome branched three times. After branching, the older portions of the rhizome decay with the advent of time and two individuals result. Such a method of reproduction is characteristic of practically all plants with an underground branching stem. Fig. 13 is a representative longitudinal view of the apex of a rhizome which is on the verge of losing its growth by means of an apical cell, in favor of growth by meristem of two or three cells. To the left of this figure is shown the origin of desmogen tissue and a possible leaf initial. The sequence of segments may be traced here to better advantage than in any other rhizome studied. It can be seen that the segmentation has been regular, and almost of the leptosporangiate type until the last three or four segments, when the apical cell was apparently thrust to one side by the overgrowth of one of the lateral derivatives. This lateral derivative then, instead of undergoing the usual periclinal division, divided in a plane perpendicular to the surface of the apex, resulting in the formation of two cells approximately equal in rank to the apical cell. Extensive studies of like situations would indicate that this is a preliminary step in the replacement of the apical cell with a meristematic group. The other

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to left; cross-section of rhizome. Fig. 3, longitudinal section of young root with definite apical cell (*a*); segments which will contribute to formation of root cap not yet formed. Fig. 4, longitudinal section of young root with definite apical cell which has cut off initial cell of root cap to left; endodermis at left. Fig. 5, cross-section of young root passing through cortex of rhizome; sequence of segments obviously irregular. Fig. 6, longitudinal section of rhizome showing enlarged root initial being formed in pericycle just within endodermis (*e*). Fig. 7, cross-section of young root entering soil; individuality of original apical cell is being lost, and a 2- or 3-celled meristem apparently taking its place. Fig. 8, longitudinal section of rhizome and young root showing truncate appearance of 5-sided apical cell, and portion of leaf trace with which vascular system of root will ultimately become continuous. Fig. 9, cross-section of apex of young rhizome just before forking, showing apical cells of the two branches. Fig. 10, cross-section of apex of old rhizome with apparently 3-celled meristem. Fig. 11, cross-section of apex of very young rhizome showing definite tetrahedral apical cell which has undergone rather definite segmentation; leaf initials not distinguishable. Fig. 12, longitudinal section of apex of young rhizome with definite tetrahedral apical cell. Fig. 13, longitudinal section of old rhizome; growth slow and individuality of original apical cell practically lost; elongated cells which later become desmogen tissue at left. Fig. 14, cross-section of apex of old rhizome; irregular triangular apical cell represented with three young leaves (*l*) and possible initial of fourth. Fig. 15, cross-section through apex of old rhizome showing one of many irregular types of meristems found in this species.

possibility remains, however, that this is merely an expression of one of the many irregularities that may be expected in the apical meristem of any of the Osmundaceae, even if a functioning apical cell be present.

Whatever the type of segmentation, lateral derivatives of the apical or master cells divide, at first periclinally, and then in three planes in such a manner as to form two or four large outer cells and a number of smaller inner cells for each segment (fig. 13). The latter elements form a solid dome-shaped structure, the peripheral members of which give rise to desmogen tissue, unbroken by leaf gaps. The larger outer cells mentioned in connection with the lateral derivatives of the apical cell undergo a few anticlinal divisions, and ultimately certain of their progeny give rise to leaf initials, while the remainder contribute to the undifferentiated epidermis of the leaf.

Root origin and development

An effort was made to determine the histological relationships of the tissue in which the root initial appears, but, due to the slow differentiation and maturation of the stem, combined with the early origin of leaf traces, decisive information was not obtained. A number of factors indicate that the young root arises in a tissue which is on the border line between the stele and cortex, and which later becomes differentiated into pericycle (figs. 1, 6). Studies in this connection indicate that this tissue may not be traced to any definite cell derived from segments cut off by the apical cell or meristematic group.

The initial cell of the root may be observed very near the tip of the rhizome. It is conspicuous on account of its large size and by its nucleus, which is usually nearly twice as large as the nuclei of the surrounding cells (fig. 1).

As in the case of the stem, development of the root is characterized by extreme irregularity. Two, or even three initial cells may be involved in the origin of the root, one of which may give rise to an apical cell, or the group may form an irregular meristem. After examination of a great number of rhizomes, the conclusion was reached that the mechanics involved in probably more than half of the cases are in accordance with the following description: Shortly

after the root initial is differentiated, it undergoes two divisions in a plane parallel, or nearly parallel to the longitudinal axis of the rhizome and perpendicular to its surface. Due to the growth of the leaf trace, with which the vascular system of the root will ultimately become continuous, the resulting cells may be slightly stretched in a plane oblique to the axis of the rhizome, a fact which makes it extremely difficult to obtain median sections, regardless of how the rhizome be oriented in cutting. The three cells obtained by the two divisions just described often undergo a period of enlargement before further divisions occur (fig. 2). The surrounding tissue continues to divide, thus keeping pace with the enlargement of the root initial and the growth of the leaf trace with which the vascular system of developing root becomes continuous.

In *Osmunda cinnamomea* the prevailing situation is one root for each leaf, but a few instances were observed in which two root initials had been formed for a single leaf; one of them, however, proceeded only to the formation of a few cells before its growth was inhibited.

After the three-celled stage the young root develops rapidly. An oblique wall is laid down in the central cell, resulting in the formation of a wedge-shaped element which functions as the apical cell of the young root. The lateral members of the original three also divide into a number of more or less rectangular cells (fig. 3). Sometimes one of these lateral cells may divide in a manner suggestive of an apical cell (fig. 4), but its activities are soon altered and subordinated if the original apical cell is to retain its identity. If these lateral cells divide and keep pace with the apical cell, a multicellular meristem results.

The apical cell of the young root just described has five cutting faces. It may be triangular in profile, or truncate at the base (fig. 8). The four posterior faces cut off segments which form the body of the root, but contribute also to the root cap, while the fifth anterior face contributes to the formation of the root cap exclusively. Fig. 8 was drawn from a longitudinal section of a young root. The apex of the root is capped by a layer of cells which are probably endodermal, but have not been derived from the apical cell of the root. Such a situation is frequently found in angiosperms, where the lateral roots

are distinctly pericyclic in origin. The layer of cells, which may be the undifferentiated endodermis, is easily distinguished at the time the root initial is set off from the rest of the tissue (figs. 1, 6). Its histogenetic relationship is not clear, as already indicated, due to the frequent interruptions of the continuity of the stele by leaf gaps. It should be noted, however, that at the time of the origin of the root initial the pericycle consists of but one layer of cells (fig. 6), and is distinct from the endodermis. Tracing such sections upward toward the apex of the rhizome has resulted in strong but not positive evidence for the independent origin of the pericycle and endodermis.

A few cases were observed in which a triangular pyramidal apical cell might have been present. The same condition has been reported for the older roots of *Osmunda regalis* by BOWER (3). In none of the cases, however, did the triangular cell function with the diagrammatic regularity so characteristic of the leptosporangiate ferns. Such apices are regarded as possessing a meristematic growing tip (fig. 7) rather than a triangular apical cell. The majority of the young roots had a five-faced apical cell, as previously described.

Segmentation may be counter-clockwise for a time (three or four segments), but as the root enlarges and starts to pass through the cortex of the rhizome, the walls seldom follow in the typical spiral succession. Fig. 5 represents a cross-section of such a root. The irregularity of placement of walls may readily be observed, and deemed doubly significant, inasmuch as, of the fifteen or twenty cases examined, this root was selected as having the most regular segmentation. Irregularity increases with age, and in old roots the single apical cell gives rise, in many instances, to a pair, three, or even a group of initials, horizontally seriated and of approximately the same rank. This irregularity, which results in the loss of the apical cell, is not confined to the root, but is also characteristic of the stem, as was shown previously. Such evidence suggests that *Osmunda* is losing its growth by means of an apical cell, in favor of a type of growth exemplified by some of the higher plants, and more characteristic of massive stems. The evidence is distinctly in support of the views set forth by BOWER (3).

There has been no satisfactory work to date on the histogenesis

of the roots of the Osmundaceae. BOWER describes the meristem of
the root of *Osmunda* as being subject to variation, and states:

since it is hardly possible to determine with certainty from longitudinal sections
what is the actual number and form of the initial cells, a full and exact inter-
pretation of the structures in longitudinal sections cannot be expected.

Most of the sections examined by the writer were in support of this
conclusion.

Discussion

Nearly a half century ago BOWER (3) made a comparative study
of the meristems of a group of families which are included with the
Filicales, namely, Hymenophyllaceae, Polypodiaceae, Cyatheaceae,
Schizaeaceae, Osmundaceae, and Marattiaceae. His chief contribu-
tion was to demonstrate a parallelism in character of all the meri-
stems in these divisions, and to show that in the first named groups
the meristems are simple in construction, consisting of an apical cell
which exhibits the definite regularity of segmentation also character-
istic of the Equisetales. Passing upward through the series, BOWER
found that regularity of segmentation is no longer characteristic of the
growing points, and that the identity of the single initial is lost as
the sequence of segments becomes less definite. The list of families
just given is taken from his paper as representing a natural series
which grades from the relatively simple to the more complex fam-
ilies of the Filicales.

BOWER regards the Osmundaceae as bridging the gap between the
leptosporangiate and eusporangiate ferns. He bases his evidence on
a study of the meristems of adult plants, and on certain structural
and developmental characteristics of the sporangia. He found that
the true leptosporangiate ferns have meristems characterized by an
apical cell, that is, by a single initial, and that the sporangium initial
is also a single cell. The Marattiaceae have meristems with many
initials, in some instances not unlike the growing tips of some mod-
ern angiosperms and gymnosperms. The sporangium arises from a
group of cells rather than from a single initial. With respect to these
characters the Osmundaceae occupy an intermediate position, with
their irregular meristems and sporangium initials.

It was thought that perhaps the evidence concerning the meri-
stems of the Osmundaceae might be better evaluated if a study of

the origin of the meristems were made, inasmuch as evidence of the ancestral condition may be found sometimes in the embryonic state. It has been stated already that the stem of the sporeling grows by means of an apical cell which segments in a more or less regular manner. This suggests the leptosporangiate type. As the rhizome ages, irregularities appear and a multicellular meristem frequently results, which resembles the stem tip of certain of the Marattiaceae, more definitely *Marattia* itself. A similar situation has been reported for *M. alata* by CHARLES (8), who describes the stems of young sporelings of this species as having a single irregular initial with an early transition to the complex meristem. The root initial prevailingly gives rise to a five-sided apical cell with five cutting faces, but occasionally a tetrahedral apical cell with four cutting faces is developed. This condition is the one found among leptosporangiate forms. As the root develops, the identity of the apical cell is lost and a type of growth appears which suggests the condition found in the Marattiaceae. Thus the evidence derived corroborates the conclusions of BOWER, and in addition suggests that the Osmundaceae are a transitional group; that they are at the present time losing leptosporangiate characteristics and assuming a type exemplified by the eusporangiate fern.

Although the evidence indicates that *Osmunda* is changing from one type of meristem to another, that is, from the leptosporangiate to the eusporangiate type, it throws no light upon the relative antiquity of the two groups. At first thought such evidence might appear to favor the earlier conception of BOWER, that the eusporangiates are the more recent of the groups, but it seems as logical to assume that the Osmundaceae may be passing from a relatively recent leptosporangiate type to the goal attained by modern angiosperms, rather than forward to the eusporangiate level.

The sequence of families listed by BOWER (3) was arranged on a basis of comparison of living representatives, and paleobotanical evidence was neglected, as he suggests in some of his later works (5). As early as 1891, CAMPBELL (6) argued in favor of the eusporangiate ferns as being the more primitive of the two groups. Since that time numerous papers have appeared, many of which have taken paleontological evidence into consideration. To review this literature would be beyond the scope of this paper, and it will suffice to say

that the evidence is now almost preponderantly in favor of the relative primitiveness of the eusporangiate forms. That the Osmundaceae are not contradictory in this respect has been indicated here.

If we may interpret embryonic structures as representing a condition of the adult extant in the past, it seems obvious that *Osmunda* is changing, in that it is apparently becoming more complex. From the description of CAMPBELL (7), *O. claytoniana* seems the least changed of the three species with respect to meristems, inasmuch as it retains the leptosporangiate characters to a greater degree, especially in the root. In this light, excluding anatomical evidence for the moment, it appears feasible to place *O. claytoniana* as the most primitive of the three species, instead of as the most advanced, as suggested by JEFFREY (12). *O. regalis* and *O. cinnamomea* appear to be at about the same phylogenetic level from the standpoint of meristems.

Thus two interpretations may be made on the evidence presented by *Osmunda*. If the Filicales are considered to the exclusion of the spermatophytes, *Osmunda* shows evidence of retrogression and of attaining the primitive eusporangiate type. If the spermatophytes are taken into consideration, *Osmunda* may be thought of as progressing and of approaching the goal exemplified by a number of the angiosperms, a view which is at present more acceptable.

Summary

1. The rhizome of *Osmunda cinnamomea*, when in the sporeling stage, grows by means of a tetrahedral apical cell. As the plant reaches maturity the segmentation of the apical cell becomes progressively less regular, in the majority of cases, until a condition is reached where the identity of the original apical cell is lost, and growth takes place by the activitity of two or more initials.

2. Cells which are destined to become desmogen tissue may be recognized in the second segment from the apical cell.

3. Due to the slow growth of the rhizome and the frequency of leaf gaps, it is extremely difficult if not impossible to refer any portion of the mature rhizome to any definite portion of the segments from the apical cell or group of initials.

4. The pericycle and endodermis apparently have independent origins, although decisive evidence was lacking. The roots arise in

tissue which later gives rise to the pericycle. The undifferentiated endodermis acts as a sort of protective layer to the young root.

5. The number of cells of most young roots is increased for a time by means of an apical cell with five cutting faces. As in the case of the stem, progressive irregularity in segmentation of this cell often results in the formation of two or more initial cells which replace the apical cell.

6. The evidence obtained is in support of the conclusions of BOWER (3, 5) that the Osmundaceae occupy an intermediate position between the leptosporangiate and the eusporangiate ferns, but it throws no light upon the relative antiquity of the two groups.

It is a pleasure to acknowledge the aid of Professor W. J. G. LAND, who suggested the problem, helped with the collection of material, and made suggestions concerning the interpretation of the preparations. I am deeply grateful to Professor E. J. KRAUS for his kind criticism of the manuscript and subject matter.

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CORDAITEAN WOOD FROM THE PENNSYLVANIAN OF MICHIGAN AND OHIO[1]

CHESTER A. ARNOLD

(WITH TEN FIGURES)

Among the petrified wood specimens in the Museum of Paleontology of the University of Michigan, two unstudied samples from Michigan and Ohio were recently found. The Michigan specimen was collected by a museum party from a quarry along the south bank of the Grand River, about one mile west of Grand Ledge, in 1925.[2] In the same quarry is a vein of coal a foot or more thick, and a few feet above is a massive sandstone layer bearing Calamites and Artisia casts. Immediately below the coal is a layer of *Stigmaria* shale, and below this and also between the coal and the upper sandstone are shales bearing numerous impressions of foliage, stems, and seeds of common Paleozoic plants. The source of the Ohio specimen is unknown, except that the label reads "Coshocton, Ohio." The specimen appears to have been in the collection for many years, possibly having been obtained by CARL ROMINGER.

But few American Paleozoic woods are known as compared with woods of similar age from other parts of the world. Among those described within recent years, probably the most outstanding are *Calamopitys americana* and *Archeopitys eastmanii*, from the base of the Waverley Shale of Boyle County, Kentucky (13). HOSKINS (5) and REED (11) have recently reported abundant petrified plant material in the Pennsylvanian coal balls of Illinois, which, when more fully investigated, are likely to make notable additions.

Probably the most widespread and abundant American Paleozoic wood is *Callixylon*. *C. newberryi* has long been known from the black shales of Ohio. In 1914, ELKINS and WIELAND (4) described *C. oweni* from the black shales of Indiana, and in 1922 HYLANDER (6)

described another species from New York. More recent investigations (1) have shown that wood belonging to that genus occurs in abundance just above the Genesee Shale in New York, where four recently described species, *C. erianum*, *C. bristolense*, *C. zalesskyi*, and *C. mentethense*, occur (1); and also in the New Albany Shale of southern Indiana (2). *Callixylon* wood is also known from Kentucky and Oklahoma. In Europe this genus has been reported by ZALESSKY (15) from Russia, and by KRÄUSEL and WEYLAND (8) from Germany.

During the latter half of the nineteenth century, DAWSON (3) described several forms from various Paleozoic horizons of eastern North America, under the generic designations of *Ormoxylon*, *Prototaxites*, *Nematoxylon*, *Aporoxylon*, *Syringodendron*, and *Dadoxylon*. *Prototaxites* (*Nematophyton* of PENHALLOW, and *Nematophycus* of CARRUTHERS), which was thought by DAWSON to be a conifer, is now considered a giant alga.

In 1900, PENHALLOW (10), in a revision of the known American species of *Dadoxylon*, attempted to bring the then existing knowledge of these forms up to date. He described seventeen American Paleozoic species from widely separated localities. One of these he designates as *Dadoxylon*, one as *Pityoxylon* (which according to THOMPSON and ALLIN (14) was misinterpreted), and the remaining fifteen as *Cordaites*. Several of the latter had previously been described by DAWSON as *Dadoxyla*. After the publication of DAWSON's work and before PENHALLOW's revision, KNOWLTON (7) referred many of DAWSON's species to *Cordaites*, since it was not known at the time that primary wood characters are essential for generic identification. Although the total number of species described by DAWSON, KNOWLTON, and PENHALLOW is large, their descriptions and illustrations are inadequate. In many instances the accounts are vague, inaccurate, and unsatisfactory for identification of newly discovered material.

Cordaites michiganensis sp. nov.

The Michigan specimen is a woody stem about 16 cm. long and 4 cm. in diameter. The large pith region is about 2 cm. across, and surrounding it is a woody zone having approximately the same thick-

ness (fig. 1). Nothing remains of the bark tissues except a layer of disintegrated tissue about 3 mm. thick, which is rather sharply delimited from the adjacent wood. Two nodes about 10 cm. apart are included in the specimen.

MINERALIZATION.—Some interesting features are shown by the mineralization of the specimen. The pith, from which all structure except a few mere traces had disappeared, was replaced by a core of sphalerite. While the prevailing color of the sphalerite is light green, certain portions are purple, suggesting the presence of manganese. These purple portions do not represent separate crystallization, because the cleavage planes, which show clearly under the microscope, pass uninterrupted from one area into the other. The wood region is filled with a mixture of iron pyrites and lime carbonate, the former being present in greater quantity (fig. 1). In some places both the cell walls and the lumina contain the pyrites. In other places the pyrites has infiltrated the walls but the lumina remain clear (fig. 4). In other places the reverse condition is shown, the lumina being filled with pyrites with the cell·walls calcified and consequently clear (fig. 5). Then in certain small areas the petrifying mineral is entirely lime carbonate, no pyrites being present. It is only in these portions that the specimen can be studied satisfactorily by transmitted light.

In some portions of the stem the cells are badly decayed. The disappearance of structure in the pith is especially unfortunate, since it not only renders a determination of its structure impossible, but the decay and replacement have obscured some of the features of the structure of the primary wood. In transverse section the secondary wood could be studied satisfactorily in the unpyritized portions. The preparation of satisfactory radial and tangential sections was difficult, owing to the combined problem of orientation of the specimen for cutting and the chance of the cut passing through a region consisting largely or entirely of poorly preserved tissue.

ANATOMY.—The stem is gymnospermous and without growth rings. The secondary wood tracheids average 35 μ in radial diameter, are regular in size and shape, and in general are square or slightly rectangular (figs. 5, 6). The square aspect may be increased by partial decay of the secondary walls.
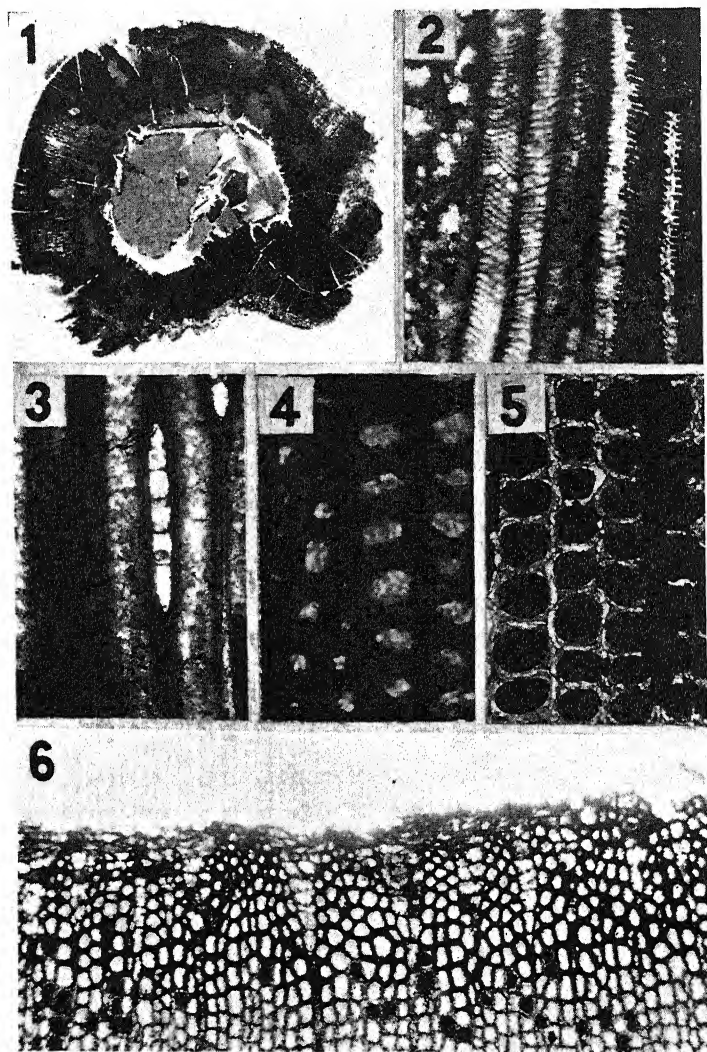
FIGS. 1–6.—*Cordaites michiganensis* Arnold sp. nov.: Fig. 1, cross-section of stem, showing dark pyritized portions and calcified areas which are lighter; ×1-3/8. Fig. 2, radial sections through primary wood showing closely wound spiral structure; lumen of tracheid at right is pyritized; ×180. Fig. 3, tangential section showing typical ray; ×180. Fig. 4, transverse section showing pyritized cell walls and calcified lumina; ×180. Fig. 5, transverse section showing pyritized lumina and calcified walls; ×180. Fig. 6, transverse section of portion of primary wood showing broadening of rays and consequent narrowing of primary wood segments as they approach pith; ×45.

So far as can be determined, the pits are confined to the radial walls of the tracheids. Decay renders them obscure, but in places of favorable preservation they are seen to be hexagonal and alternately arranged. They occur mostly in two rows per tracheid, but sometimes there are three or four.

The rays, which are small and narrow, vary in height from two to ten cells (fig. 3). For the most part they are uniseriate, but occasional partial biseriation near the middle of the ray is caused by two cells lying side by side. The biseriation seldom or never occurs for their entire height. The ray cells may be slightly higher than wide but the difference is slight, and they are variable in length, ranging from the cross diameter of one to that of four tracheids. The cross diameter of a ray cell is slightly less than that of a tracheid.

As the rays approach the pith they gradually broaden, becoming narrowly fan-shaped. Consequently the xylem masses between these rays become narrowed to a point at the margin of the pith (fig. 6). These narrow wedges of the inner wood represent the primary xylem, which in this case is not sharply defined from the secondary wood to which it is joined. At the tips of these wedges the xylem elements are small, but they become progressively larger farther back. At the broadest place the wedges vary in width from one to seven or eight cells. These wedge-shaped masses consist of tracheids of the closely wound spiral type (fig. 2), which extend radially for a distance of eight to ten tracheids, as near as preservation of the material permits one to judge, and in some places a brief zone of one or two cells shows reticulated structure just before pitting commences.

Because of pyritization and decay at several critical places, little can be determined concerning the origin and departure of the traces, although they appear in some of the more favorable instances to consist of a double strand. Consequently it is impossible to determine with any degree of certainty whether they possess any centripetal primary wood. For this reason it is unadvisable to refer this form to *Mesoxylon*, which, according to MASLEN (9), is to be distinguished from *Cordaites* only by the presence of centripetal wood on the downward extension of the leaf trace at the margin of the pith.

In default, therefore, of this one character which is essential in

identifying a form as *Mesoxylon*, and because of the close resemblance of this specimen to forms originally described as *Cordaites*, it is considered advisable to refer it to that genus. According to RENAULT (12), the primary xylem region of *Cordaites* consists of a series of inward projecting wedges separated one from the other by the broadening of the rays as they approach the pith. This feature was also observed in *Mesoxylon multirame*, when comparing my specimen with a specimen of that species. MASLEN states that possibly the majority of the stems previously described as *Cordaites*, have the *Mesoxylon* structure, suggesting the possibility of RENAULT's *Cordaites* being a *Mesoxylon*. The centripetal wood might easily have been overlooked, since at the time he wrote the primary wood was not yet regarded as important for generic identification. At any rate, RENAULT's *Cordaites*, *Mesoxylon*, and my form show marked similarity in primary wood structure, and the apparent differences might be due to preservation. RENAULT's description of the wood of *Cordaites* is brief and lacks many desirable details. Also it should be noted that the distinguishing feature of *Mesoxylon* is merely the presence of a structure not mentioned by RENAULT and which he probably overlooked. On the other hand, it is true that during Paleozoic times the cordaitalean complex was a large and diversified group of plants, and the occurrence of two forms differing only with regard to the feature under consideration is easily within the range of possibility.

The large pith (whether or not it was discoid could not be determined), the alternate-multiseriate pitting, and the narrow rays are features also indicative of *Cordaites*.

The Michigan wood does not seem to agree closely with any of the American cordaitean forms described by DAWSON (3) or PENHALLOW (10). It appears to differ in many respects from *Cordaites brandlingii*, the common European form, and it is unlikely to have affinities with the numerous forms from the upper Coal Measures or Permian formations. Since this specimen is older than most of the other known forms (it being from the lower Coal Measures), and because of its isolation (the nearest previously described forms are from Illinois and Ohio, and they differ from it), it appears advisable to describe it as a new species.

Cordaites michiganensis sp. nov.—Tracheids averaging 35 μ in diameter, square to slightly oblong, regular in size and shape. Bordered pits alternately arranged, hexagonal, chiefly in 2 rows per tracheid. Rays narrow, low, and scattered; uniseriate or partly biseriate in the middle. Ray cells about equal in height and width, narrower than the tracheids, in length equal to the cross diameter of 1–4 tracheids. Rays broader as they approach the pith, dividing the primary wood region into wedge-shaped segments.

Horizon, probably Saginaw, lower Pennsylvanian. Locality, Grand Ledge, Michigan. Type no. 12405, Museum of Paleontology, University of Michigan.

Dadoxylon romingerianum sp. nov.

The Ohio specimen consists of a piece of well preserved silicified secondary wood measuring about 2.5×5×8 cm. Judging from the convergence of the radial faces of the block it apparently came from a large trunk, at least 6 inches from the center. Since there is no information concerning the source of this specimen, other than the label which reads "Coshocton, Ohio," its Pennsylvanian age is only inferable.

The wood, which is gymnospermous (fig. 7), shows no growth zones in the 5 cm. of its radial extent. The tracheids are large, averaging 60–70 μ in radial diameter, but varying from 45 to 90. The radial diameters are somewhat greater than the tangential diameters, which are variable. Some of the tracheids are nearly round, with all diameters approximately equal.

The bordered pits are alternately arranged in 2–5 rows per tracheid. Two or three rows are most commonly observed (fig. 8). Tracheids bearing five rows of pits are rather rare. The pits are of the hexagonal type but are slightly compressed vertically, thus rendering the vertical diameter somewhat less than the transverse one, which varies from 12 to 18 μ (fig. 10). The pit orifice is the diagonal slit commonly observed in woods of this type, but because of the narrowed vertical diameter of the pit the orifice is inclined at an angle somewhat less than 45° from the horizontal. In some of the more closely crowded pits the orifice lies nearly at right angles to the vertical axis of the tracheid.
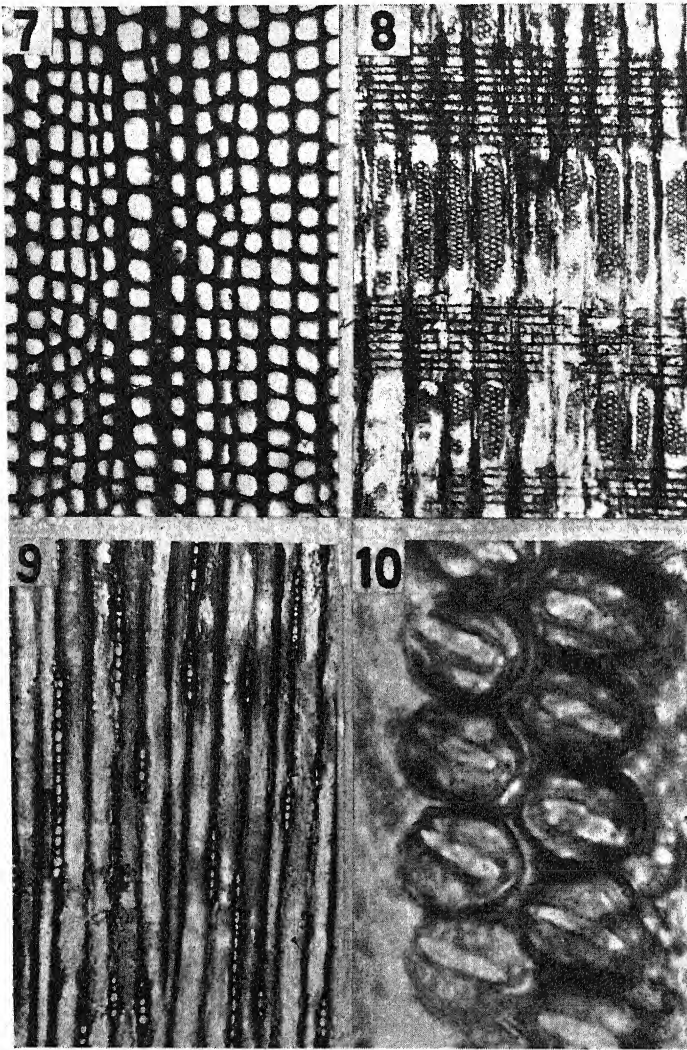
FIGS. 7–10.—*Dadoxylon romingerianum* Arnold sp. nov.: Fig. 7, transverse section; ×55. Fig. 8, radial section showing characters of rays and pitting; ×65. Fig. 9, tangential section showing narrow rays in contrast to broad tracheids; ×65. Fig. 10, bordered pits in radial section, highly magnified, showing slight vertical flattening of bordered pits and low inclination of pit orifice; ×1300.

The rays, most of which are less than twenty cells high, may vary from two to forty cells (fig. 9). They are mostly uniseriate but partial biseriation occurs frequently. The biseriation may extend vertically for about six cells in some instances, but ordinarily it consists merely of two cells lying side by side near the middle of the ray. The ray cells are thin-walled and some of them have dark contents. The side walls are pitted, there being usually one or two pits between the ray cell and each adjacent tracheid. The ray cells range from 15 to 18 μ in width, with their vertical and horizontal diameters about equal. In length they are approximately equal to the cross diameters of 4–6 tracheids. The ray cells of small diameter in connection with the large tracheids provide a characteristic which is obvious and appears to distinguish this form (fig. 8).

In the absence of the primary wood the exact generic affinities of this specimen are indeterminable, and must remain an open question, so it is considered well to adhere to the established custom of referring such problematic material to *Dadoxylon*. While showing the same general *Dadoxylon* (or cordaitean) type of structure as the Michigan specimen just described, the two specimens are specifically different, and aside from very general features there are no indications of their generic identity. Several forms, of which *Mesoxylon*, *Callixylon*, and *Poroxylon* are examples, are cordaitean and would probably be considered congeneric were it not for the distinctive characters of the primary wood.

The characters which serve to distinguish the specimens may be contrasted thus:

CORDAITES MICHIGANENSIS	DADOXYLON ROMINGERIANUM
Tracheids regular, average diameter 35 μ.	Tracheids irregular, average diameter 60–70 μ.
Bordered pits mostly in 2 rows, height and breadth equal.	Bordered pits mostly in 2 or 3 rows compressed vertically.
Rays low, seldom more than 10 cells high.	Rays higher, frequently more than 10 cells high.
Rays only slightly narrower than tracheids.	Rays much narrower than tracheids.

In cell dimensions, biseriation, etc., the rays of the two specimens are similar. As noted, however, they are somewhat higher in the

Ohio specimen, and being associated with rather large tracheids the ray cells appear small in proportion, a feature not so obvious in the Michigan form.

The Ohio specimen, in comparison with forms previously described from Ohio, shows little evidence of near affinity. Perhaps the closest approach is *Cordaites matariarum* Daws., which, according to PENHALLOW (10), has tracheids $45 \times 70 \mu$ and narrow rays. The pits of this form, however, do not show the vertical shortening which is such a conspicuous feature in our specimen. *Cordaites ohioense* Daws. (10) and *Callixylon newberryi* (Daws.) Elkins & Wieland (4) show no basis for comparison. In describing this specimen as a new species it is certainly appropriate to name it in commemoration of the well known geologist of the last century, CARL ROMINGER, in whose wood collection this specimen was found.

Dadoxylon romingerianum sp. nov.—Tracheids averaging 60–70 μ in diameter, irregular in size and shape. Bordered pits alternately arranged in 2–5 (mostly 2–3) rows per tracheid, compressed vertically, horizontal diameter 12–18 μ; orifice a diagonal slit. Rays narrow, few to 40 cells high, uniseriate or partly biseriate in the middle. Rays long, height and width about equal, much narrower than adjacent tracheids. Pits on side walls of ray cells 1 or 2 per tracheid.

Age, probably Pennsylvanian. Locality, Coshocton, Ohio. Type no. 12406, Museum of Paleontology, University of Michigan.

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SPECIALIZATION IN SECONDARY XYLEM OF DICOTYLEDONS

III. SPECIALIZATION OF LATERAL WALL OF VESSEL SEGMENT

FREDERICK H. FROST[1]

(WITH EIGHT FIGURES)

Introduction

In 1894 BOODLE and WORSDELL (4) advanced the hypothesis that scalariform lateral pitting in the vessels of dicotyledons originates by fusion of horizontal rows of circular pits. In recent years JEFFREY (9), BLISS (3), and MacDUFFIE (10) have elaborated a similar concept. On the contrary, THOMPSON (11), BROWN (5), BAILEY and TUPPER (2), and BAILEY (1) have contended that scalariform lateral pitting is primitive and gives rise by specialization to the circular type. In view of the fact that both groups of investigators agree that the transitional types of pitting between these two extremes are phylogenetically significant, the main point at issue is the problem of determining the direction of the specialization. This paper attempts to settle this question, based on a study of a wide range of dicotyledons and by evidence independent of the transition itself. The nature of the pitting between vessels and parenchyma is also discussed.

LATERAL INTERVASCULAR PITTING

The type of pitting between adjacent vessel segments is divisible into four classes: scalariform (fig. 1), transitional (fig. 2), opposite (fig. 3), or alternate (fig. 4). Scalariform lateral pitting is characterized by the oblong-elongate form of the pits in surface view. Transitional lateral pitting, as the name implies, is a combination of scalariform and opposite pitting. Scalariform and transitional lateral pitting occur in many families of dicotyledons. The scalariform type is not a "phenomenon of rare occurrence" BLISS (3). Opposite pitting is distinguished from alternate by the orientation of the pits.

In the former the pits are arranged in horizontal rows, whereas in the latter they form diagonal (but not horizontal) rows of contiguous pits. Opposite pits tend to be oval or rectangular, and alternate ones round or hexagonal; both types are classified under the heading of "circular" pits. Circular pitting characterizes the vessel segments of about 80 per cent of the existing species of dicotyledons.
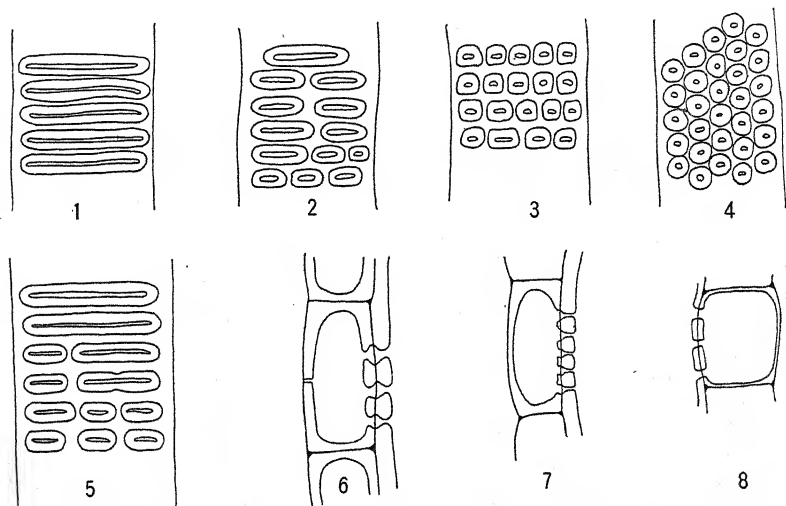
Figs. 1–8.—Fig. 1, scalariform lateral pitting from *Hedycarya arborea;* fig. 2, transitional lateral pitting from *Talauma ovalis;* fig. 3, opposite lateral pitting from *Liriodendron tulipifera;* fig. 4, alternate lateral pitting from *Planera aquatica;* fig. 5, transitional stages in formation of circular pitting from *Magnolia kobus;* fig. 6, fully bordered vessel-ray pits from *Sassafras variifolium;* fig. 7, half-bordered vessel-ray pits from *Hicoria ovalis;* fig. 8, non-bordered vessel-ray pits from *Bombax malabaricum.*

It was shown in the first (7) and second (8) papers of this series that specialization of the vessel results in a gradual reduction in length of the vessel segments. By the application of the method of correlation, if circular pitting is more primitive than scalariform, it follows that the vessel segments which are characterized by circular pitting will be of greater average length than the scalariform-pitted segments. On the contrary, if the scalariform type of pitting is primitive the reverse will be the case. Table I shows the actual correlation.

The numerical difference between the means of the scalariform

and transitional groups is not statistically significant, due to the small number of cases and the relatively slight distinctions between the two modes of pitting. The differences between the means of the scalariform and transitional groups and the opposite group, however, is statistically significant since a chance difference of this magnitude would not occur once in ten thousand times. The mean of the alternate group is likewise stable. The obvious conclusion is that scalariform lateral intervascular pitting is primitive, and that the sequence should be read from scalariform, to transitional, to opposite, culminating in the highly specialized alternate arrangement of circular pits.

It has been shown in table IV of the first paper (7), and by BAILEY and TUPPER (2), that the stages of the transition from scalariform to

TABLE I

Type of lateral pitting	No. of species	Length of vessel segments (mm.)
I. Scalariform	15	1.13
II. Transitional	28	1.07
III. Opposite	33	0.79
IV. Alternate	183	0.46

simple perforations correlate with the stages of the transition from scalariform to alternate intervascular pitting. This is an important fact, since the followers of the fusion hypothesis hold that scalariform perforation is ancestral to the simple perforation. If the fusion hypothesis is still maintained despite this evidence, therefore, it will be necessary either to abandon the concept that the scalariform perforation is more primitive than the simple perforation, or to admit that the characteristics of a cell type or homogeneous tissue can develop independently of one another. If the existence of phylogenetic correlation is denied, in the face of the many proofs of its reality, the solution of the problem becomes an impossibility. It should be noted that the doctrines of recapitulation and of conservative regions would be invalidated if phylogenetic correlation between tissues were non-existent.

Palingenetic sequences from scalariform to opposite pitting are frequent, and may be found in the variability presented by the

species of a genus, the variability of the vessel segments of a species, in the transitional region between the protoxylem and the secondary xylem of many of the less specialized dicotyledons, and not uncommonly on the lateral walls of an individual vessel segment. Fig. 5 (*Magnolia kobus*) illustrates the breaking down of scalariform pitting as reflected in positional sequence on the lateral wall of the vessel segment. In the majority of dicotyledons this transition has reached the alternate arrangement, since the alternate condition is by far the most common type. This is particularly true of species found in temperate regions.

As the vessel segment is reduced in length during specialization, it frequently increases in width. In general the breaking down of the scalariform intervascular pitting occurs before or in correlation with this increase in diameter of the vessel. Occasionally, however, the scalariform sculpture persists and in such instances the scalariform pits are apparently drawn out horizontally. *Rhizophora mangle*, *Bruguiera gymnorrhiza*, and *Vitis* show this condition. The vessels are large in cross-section and show decided evidences of specialization. The lateral pitting has remained scalariform, however, and the pit orifices are narrow.

VESSEL-PARENCHYMA PITTING

The type of pitting found between vessels and parenchyma is not, as has been suggested (9), controlled by the orientation of the parenchymatous elements. In the majority of cases the pitting found between parenchyma and vessels is similar in type to the intervascular pitting of the species under consideration. That is, the pits may be scalariform, transitional, opposite, or alternate. In the 260 species studied in this connection, it was found that in 85 per cent the vascular-parenchyma pitting was of the same type as the intervascular pitting. As a rule scalariform and transitional vessel-ray pits are oriented with their long axis parallel to the direction of the ray. In some species of *Quercus* and *Castanea*, however, the long axis is vertical. Transitional types between the horizontal and vertical position are not infrequent. The correlation between vessel segment length and the nature of the vessel-ray pits is shown in table II.

The correlation between specialization in the length of the vessel

segment and the evolutionary development of the vessel-ray pitting
is even more striking in this case than the correlation shown in
table I. The average length for each type of pitting, however, is
slightly lower for each group in table II. This difference is probably
significant, since the intervascular pits specialized more rapidly than
the vascular-parenchyma pits, as indicated by the fact that in 85
per cent of the species, which show a difference in pitting in these
two situations, the intervascular pits are more highly specialized
than the vascular-parenchyma pits. This slightly slower rate of de-
velopment would tend to reduce the means in each case.

The situation in *Trochodendron* and *Tetracentron* is interesting in
this connection. In both of these vessel-less genera the first formed
tracheids of the annual ring have the primitive scalariform lateral

TABLE II

VESSEL-RAY PITTING	NO. OF SPECIES	LENGTH OF VESSEL SEGMENTS (MM.)
I. Scalariform..............	13	1.11
II. Transitional..............	42	0.96
III. Opposite..................	49	0.74
IV. Alternate.................	156	0.43

pitting. Where tracheids of this type adjoin ray parenchyma the
pitting is transitional to opposite; scalariform pits are found near the
pith but are of rare occurrence in the later growth rings. The type
of pitting found between the circular pitted tracheids and the ray
parenchyma is opposite or alternate, as one would expect. It is ap-
parent that the specialization of the tracheid-ray pits proceeded
more rapidly than the specialization of the tracheid pitting, the re-
verse of the usual procedure when the ray is in contact with a vessel
segment.

The writer (6) has shown that vessel-parenchyma pits may be
fully bordered, half-bordered, or non-bordered (figs. 6–8).

Correlating these types with an established evolutionary sequence
in order to determine the course of development presents several
difficulties. In well prepared radial and tangential sections it is im-
possible in many cases to find more than three or four pits which can
be classified definitely, and in such instances the dominant type can-

not be determined. In other cases the vessel-ray pits are so minute that it is unsafe to reach a decision as to their nature. While these difficulties have made it impossible to work out the details of the evolutionary development of the bordered pit, it has been possible to determine certain facts regarding the specialization. Table III shows the correlation between the nature of the border and the length of the vessel segment.

TABLE III

Type of border	No. of species	Vessel segment length (mm.)
I. Bordered..................	58	0.81
II. Half-bordered.............	170	0.56
III. Non-bordered.............	23	0.53

The correlation between the type of border and the type of vessel-parenchyma pit is given in table IV. If this evidence is assumed to be statistically reliable, the direct interpretation is that the fully bordered pit is primitive and that the sequence should be read from

TABLE IV

Type of pitting	No. of species	Percentage of species with fully bordered pits
I. Scalariform and transitional..	54	46
II. Opposite.................	49	35
III. Alternate.................	147	11

fully bordered, to half-bordered, to simple or non-bordered. Since it is relatively easy to distinguish the species possessing half-bordered and fully bordered vessel-parenchyma pits from those with simple pits, it is clear that the non-bordered condition is a specialization of either the bordered or half-bordered pit. This is indicated by the fact that the average length of the vessel segments of species with fully bordered and half-bordered vessel-parenchyma pits is higher than the average for species with simple pits, since the non-bordered type is not found in the truly primitive woods, and since primitive plants in general are characterized by the half-bordered or bordered pit in this situation.

The relative position of the bordered and half-bordered types is not so clear, since the separation into half-bordered and bordered groups cannot accurately be accomplished. Although tables III and IV indicate that the fully bordered pit is the most primitive, there are undoubtedly fully bordered species listed as half-bordered and half-bordered species listed as fully bordered. In addition to this complication, the specialization of the bordered pit is not highly correlated with the development of other characteristics of the vessel segment. This results in woods with bordered and half-bordered vessel-parenchyma pits which are highly specialized in other respects.

The most probable interpretation of these data is that the pro-angiospermous stock possessed bordered pits in this situation and that the fully bordered type persisted after the introduction of the vessel. Soon after, the half-bordered type originated by the loss of the border on the wall of the living cell, and later the loss of the remaining border produced the non-bordered vessel-parenchyma pit. A delay in the introduction of the half-bordered pit, possibly due to environmental factors, would then account for the highly specialized woods with fully bordered pits.

Another alternative is that the half-bordered pit is primitive and was characteristic of the pro-angiospermous stock. If this were the case the bordered pit must have originated practically simultaneously with the vessel, since the fully bordered type is characteristic of many of the more primitive existing dicotyledons. Subsequent specialization of the bordered pit might then produce the half-bordered and non-bordered types, or the simple pit might originate directly from the primitive half-bordered pit. While this explanation is possible it does not appear very probable.

Unfortunately the preservation of the older fossils with scalariform tracheids is not such as to allow a distinction to be made between the bordered and half-bordered vessel-parenchyma pitting, and at the present time the true nature of these pits is doubtful. The problem will therefore remain unsettled until satisfactory palaeontological material establishes which of these alternatives is the correct interpretation.

The question of tertiary spiral thickenings is of interest. Of the

260 species studied, 41 had spiral thickenings in the vessels of the secondary wood. The average length of the vessel segments of these 41 species is 0.46 mm., which is less than the average length of vessel segments in general, and shows that introduction of the spiral is an evidence of decided specialization. Spirals normally occur in woods with porous perforations, but may be found occasionally in scalariform and scalariform-porous woods (*Magnolia*). In the latter case the vessels always show several markedly specialized features. The vessel segments are short, the lateral pitting opposite, the apertures of the perforation non-bordered and large, the number of bars reduced in number, or the walls considerably thickened. The writer has not observed spirals in such primitive woods as *Schima wallichii* or *Dillenia philippinensis*.

Summary

1. Scalariform intervascular pitting is primitive in the organization of the vessel segments of the dicotyledons.

2. Specialization of the scalariform pit produces transitional and opposite pitting. The re-arrangement of opposite pits gives rise to the highly specialized alternate arrangement of intervascular pits.

3. Vessel-parenchyma pitting may be scalariform, transitional, opposite, or alternate. The evolutionary development is the same as in the development of intervascular pitting.

4. Vessel-parenchyma pits may be fully bordered, half-bordered, or non-bordered. The fully bordered pit appears to be primitive and to give rise during specialization to the half-bordered and the non-bordered type.

5. The introduction of tertiary spirals in secondary vessel segments is an evidence of specialization. Spirals do not occur in the most primitive dicotyledonous woods.

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DEVELOPMENT OF FLORAL ORGANS
OF THE SOY BEAN[1]

ARTHUR T. GUARD

(WITH SIXTEEN FIGURES)

Development of the floral organs of *Soja max* Piper has been studied, from the appearance of the knoblike protuberance which forms the receptacle to the mature flower, in order to determine the sequence of appearance of the organs and the course of their development to maturity. Only the Manchu variety was used. The plants which furnished material for this investigation were grown in the garden, since attempts to grow material in the greenhouse yielded only poorly developed buds and flowers which never fully opened.

Rawlin's formula of formalin-acetic-alcohol, Schaffner's chromo-acetic acid, and Flemming's medium solution were employed as killing and fixing agents. Either Delafield's haematoxylin counterstained with safranin or Flemming's triple was used as the stain.

GROSS MORPHOLOGY

The flowers of the soy bean are borne in axillary (rarely terminal) racemes (fig. 1), usually of five to sixteen flowers each. PIPER and MORSE (4) report instances of as many as thirty-five flowers in a single inflorescence. The perfect, polypetalous, zygomorphic flower is approximately 6 mm. in diameter when fully opened.

The calyx is tubular, terminating in five unequal lobes of which the largest is anterior, the next two lateral, and the two smallest obliquely posterior. It is persistent, but grows little during the development of the fruit. The corolla consists of five distinct petals (fig. 4). The largest is posterior, the two next in size (wings) are lateral, and the two keel petals are obliquely anterior. Although the margins of the two keel petals touch, there is no coalescence, as is the case in the keel petals of the flowers of many legumes.

The androecium consists of ten diadelphous stamens (fig. 2), all

[1] Contribution from the Biology Department of Purdue University, Lafayette, Indiana.

of which are separate at first, but later the filaments of nine of them are elevated as a single structure by the pushing up of a basal region of meristematic tissue, leaving the posterior stamen separate. The single pistil (fig. 3) is already covered with trichomes by the time the flower opens. These are of two kinds, one (fig. 15) a unicellular solitary structure, the other (fig. 14) a multicellular uniseriate trichome usually of four cells, occasionally of five or six. The style, which is about half the length of the ovary, curves backward toward

FIG. 1.—Inflorescence of soy bean, a raceme (usually axillary, occasionally terminal)

the posterior stamen, and is surmounted by a knoblike stigma which is receptive for pollen at the time the flower opens.

EMERGENCE OF ORGANS

The first sign of a flower in the soy bean is the appearance of a knoblike primordium in the axil of a bract (fig. 5) before this has reached its mature size. The anterior calyx lobe arises on the anterior side of this primordium (fig. 6), followed by the two lateral lobes, and later by the two obliquely posterior lobes (fig. 7). Early in their development the bases of the calyx lobes broaden until they meet, and finally the whole basal region is pushed up as the calyx tube (fig. 8). The primordia of the petals (fig. 9) are alternate with the lobes of the calyx, and more distal on the floral axis. The two obliquely anterior or keel petals appear first, next the two lateral ones (wings), and finally the posterior petal. This sequence of emergence
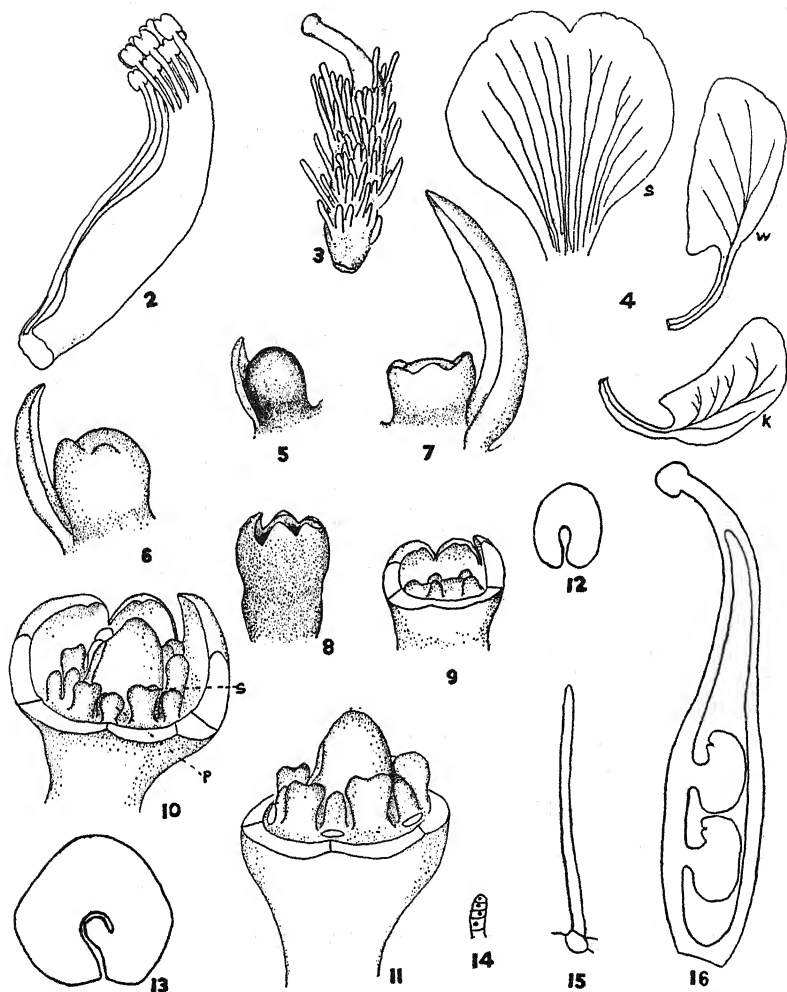
FIGS. 2–16.—Fig. 2, Androecium showing nine united and one free filament; ×10. Fig. 3, pistil with ovary densely covered with trichomes as it appears as flower opens; ×10. Fig. 4, part of corolla; ×10. Fig. 5, young flower primordium appearing in axil of bract; ×90. Fig. 6, flower primordium with calyx lobes beginning to emerge; ×90. Fig. 7, flower primordium with only primordia of calyx lobes present (no other parts yet appeared); ×90. Fig. 8, entire calyx region pushing up to form calyx tube; ×90. Fig. 9, flower primordium with part of calyx cut away to show five petal primordia just emerging; ×150. Fig. 10, more advanced stage than fig. 9, with part of calyx cut away to show primordia of petals (p), stamens (s), and pistil; ×200. Fig. 11, flower bud with calyx and petals cut away to show position and relative size of two cycles of stamens and

corresponds with that observed by PAYER (3) in *Trifolium ochroleucum*. GOEBEL (2) reports this to be the usual order of emergence of floral organs in the Leguminosae.

While the petal primordia are still very small, the primordia of the outer cycle of stamens (fig. 10) appear. The five individual members of this cycle are opposite the calyx lobes and alternate with the petals. The sequence of appearance is also from anterior to posterior on the receptacle.

By the time the last primordium of this cycle is visible, a second cycle of stamens, its members alternating with those of the first, begins to develop in the same order. That they are slightly distal to the first cycle is noticeable in the early stages of development (fig. 11), but later the inner cycle appears to merge with the outer, as a result of the development of the tissue which is basal to both cycles. The presence of two cycles of stamens, one slightly preceding the other, was also observed by PAYER (3) in *Trifolium ochroleucum*, WESTGATE and his co-workers (5) in *Trifolium pratense*, and COE and MARTIN (1) in *Melilotus alba* and *M. officinalis*. Almost simultaneously with the emergence of the last cycle of stamens, the single primordium of the carpel appears. This develops rapidly and early becomes the largest of the primordia (fig. 11).

GROWTH OF ORGANS

Before there is any differentiation in either stamens or pistil, the calyx during its development forms a protective covering for the other organs. At this time the trichomes are beginning to form on the calyx, and these continue to increase in number until the calyx is very hairy. These trichomes are similar to those on the pistil and leaves. The growth of the petal primordia is very slow until the microsporangia begin to develop in the anthers, when the petals begin to grow rapidly, pushing the calyx lobes apart, and the flower opens.

pistil; $\times 200$. Fig. 12, diagrammatic cross-section of young pistil with margins not yet coalesced; $\times 90$. Fig. 13, diagrammatic cross-section of pistil showing ovule beginning to develop on one margin; $\times 150$. Fig. 14, multicellular trichome from ovary; $\times 90$. Fig. 15, unicellular trichome from ovary; $\times 90$. Fig. 16, diagrammatic vertical section of pistil of mature flower showing position and orientation of ovules.

Early in its development each of the stamens is differentiated into an anther and a filament. The filaments continue to develop separately until the tetrads are beginning to round into microspores. At this time the tissue basal to the two cycles pushes up nine of them as a single structure, leaving the tenth stamen free. This free filament elongates, however, so that its anther remains on a level with the anthers of the other four stamens of the inner cycle (fig. 2). Dehiscence of the anthers occurs at the time the flowers open.

The pistil early in its development is a leaflike carpel (fig. 12), folded along its midrib with its margins scarcely touching. Before these margins coalesce, protuberances (which later develop into ovules, fig. 13) are produced alternately on the inner surface of these carpel margins. In its development the pistil lags somewhat behind the stamens, as WESTGATE and his co-workers report for *Trifolium pratense*, and COE and MARTIN found in *Melilotus alba* and *M. officinalis*.

The ovules are campylotropous (fig. 16) at maturity, with the micropyle directed toward the distal end of the ovary. COE and MARTIN found a different orientation of the ovules in *Melilotus alba* and *M. officinalis*, in which the micropyle is facing the basal end of the ovary. There are usually two or three ovules in each ovary, but occasionally only one or rarely four are found. These ovules develop simultaneously. The style grows slowly until the flower is ready to open, when it elongates rapidly until it is about two-thirds the length of the ovary. The stigma is a knob covered with papillae.

Summary

1. The mature flower of the soy bean consists of a calyx tube with five unequal lobes, five distinct petals, ten stamens (nine united and one free), and a single carpel. Both unicellular and multicellular trichomes are found on the calyx and pistil.

2. The flower primordium appears first as a protuberance in the axil of a bract. The order of emergence of the floral organs is sepals, petals, outer cycle of stamens, inner cycle of stamens, pistil. The order of emergence within each cycle is from the anterior to the posterior side of the receptacle.

3. Development of the petals is slow at first, but later becomes

rapid. The stamens are free at first; later nine become united leaving one of the inner cycle free.

4. The one to four campylotropous ovules develop simultaneously, and the micropylar end is directed toward the distal end of the ovary.

The writer wishes to express grateful appreciation to Professor E. J. KOHL and to Dr. M. W. GARDNER for their suggestions and criticisms during this investigation and preparation of the manuscript.

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BRIEFER ARTICLES

A NEW SPECIES OF GRAMA GRASS

(WITH FOUR FIGURES)

Among a number of grasses which were sent to the writer by Mr. BURT ENGLISH of Fort Sill, Oklahoma, for identification was a strange specimen of *Bouteloua*. A search of literature on the genus failed to reveal a description of it. In many respects it resembles *Bouteloua hirsuta* Lag., and in the National Herbarium it has been classified as that, but the descriptions of the two grasses are not the same. The key in GRIFFITHS'[1] work on grama grasses has no place for it. His description of *B. hirsuta* does not fit, and drawings of the same species (p. 374, fig. 34) do not conform to it.

VASEY perhaps recognized this as a variety of *Bouteloua hirsuta*, but it was never validly published. Specimen no. 883032 of the National Herbarium is labeled *Bouteloua oligostachya* var. *major* Vasey, *E. P. Stiles*, Austin, Texas, 1884. This was misidentified and had been filed under *B. hirsuta*. On the mount there are the drawings of some spikelet parts, among which is a drawing of a sterile pedicel with a tuft of hair on one side at the top. The specimen on this mount is similar to the one sent in by Mr. ENGLISH. The drawing, however, should have shown a tuft of hair on each side at the top of the sterile pedicel. VASEY[2] shows a drawing of *Bouteloua hirsuta* var. *major*, but this drawing is not of the same kind of grass as that collected by *Stiles*.

Since none of the drawings describe the plant, and since there is no heretofore published description of it, it seems proper to describe it. The differences between this plant and other members of the genus appear to be sufficient to make it a new species (figs. 1–4).

Bouteloua pectinata sp. nov.—Perennis. Culmi fertiles 4–7 dm. alti, erecti, simplices, glabri; culmi steriles breves numerosique; folia plurime basalia; vaginae glabrae ac laeves, fauce ciliatae, inferiores internodiis longiores, laxae, striatae; ligula anulus ciliatus non perspicuus; laminae usque ad 25 cm. longae, 1–2.5 mm. latae, planae vel apicem versus invo-

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[1] GRIFFITHS, D., The grama grasses. Cont. U. S. Nat. Herb. 14:372–375. 1912.

[2] VASEY, GEO., U.S. Dept. Agric. Div. Bot. Bull. 12. 1890.

luto-attenuatae, erectae, flexuosae, rigidulae, margines versus crassatae, glabrae; margines minute scabrae, laminarum inferiorum basim versus papilloso-hispidae; spicae 3 vel 4, 3–5 cm. longae, leviter curvatae, pedunculo pubescenti 4–5 mm. longo gestae; rachis spicula rudimentaria terminans; spiculae 40–60, pectinatae, 5–6 mm. longae; glumae lanceola-to-acuminatae, uninerviae, inaequales, puberulae, prima 4 mm. longa nervo scabra, secunda 5–6 mm. longa (arista brevi adjecta) tergo pilis circ. 2 mm. longis perspicue papilloso-hirsuta, papillis longe arista extendentibus; lemma aristis adjectis circ. 6 mm. longa, tergo hirsuta, triaristata; palea circ. 4 mm. longa, binervia, nervis in aristas breves desinentibus; pedicellus sterilis apice crinibus bicristatus.

Figs. 1–4.—*Bouteloua pectinata:* glumes, palea, lemma, and sterile pedicel with rudimentary spikelet showing tufts of hairs at top of pedicel.

This species resembles *Bouteloua hirsuta* more closely than any other member of the genus, but differs from it in that it is a larger and more robust plant with longer spikes and more spikelets. The rachis ends with a rudimentary spikelet and the sterile pedicel has two tufts of hair at the upper end.

Bouteloua hirsuta possibly originated from *B. pectinata* by sterilization of the outer end of the rachis and disappearance of the tufts of hair at the top of the sterile pedicel. In this change it also became a smaller plant.

Specimens examined: Fort Sill, Oklahoma, *English*, 1929; Indian Territory, *Sheldon*, 1891; Fort Worth, Texas, *Tracy*, 1902; Texas, *Nealley*, 1889; Weatherford, Texas, *Tracy*, 1902; Texas, *Germey;* Fort Worth, Texas, *Ward*, 1877; Austin, Texas, *Stiles*, 1884; Wood County, Texas, *Reverchon*, 1903; West Texas, *Mohr*, 1889; Guadalupe Mountains, *Bailey*, 1901; Mesa, Texas, *Hitchcock*, 1915; Texas, *Lindheimer;* Fort Worth, Texas, *Ruth*, 1910.

The type is in the U.S. National Herbarium, no. 1445571, collected on dry soil in a rocky mountain meadow in the Wichita Mountain region

near Fort Sill, Oklahoma, August 17, 1929 by *Burt English* (no. 71). It is somewhat immature.

NATIVITY OF THE CUCURBITS

(WITH THREE FIGURES)

DE CANDOLLE, in discussing the origin of the pumpkin, stated that "historical data do not gainsay the opinion of an American origin, but neither do they adduce anything in support of it." This statement, while no doubt true at the time it was written, is no longer correct. Thanks to the archeologists, most convincing evidence bearing upon the nativity of the cucurbits has come to light, through the recoveries made in the southwest. The writer has been privileged to make a critical study of these collections, and finds that two species of cucurbits are represented. They contain numerous fragments of the rinds and peduncles. Also in mortuary bowls recovered, cucurbit seeds have been found.

PAUL S. MARTIN, in explorations made for the Colorado State Historical Society in 1928, recovered seeds from a mortuary bowl at Ackmen, Colorado, which are identified by the writer as *Cucurbita moschata*. The seeds, although well preserved, are exceedingly fragile and contain an inert brownish mass of what was formerly the embryo. The taxonomic characters of the seed coat are well defined, and clearly show the characteristic dark, tapering, wavy margin of *C. moschata*. In the Colorado State Historical Society Museum also are cucurbit fragments, collected by A. WETHERILL. The rind is charred on one edge but is otherwise well preserved It carries the characteristic green and white stripes, and also the typical neck shape of the striped cushaw, a form of *C. moschata*. The identity of the peduncles, if considered alone, might raise a question as to whether they are *moschata* or *pepo*. The peduncle of *moschata* is extremely variable, however, and from the evidence, taken as a whole, they are probably *C. moschata* also.

Through the courtesy of Messrs. KIDDER and GUERNSEY, the writer was privileged to study their collection now in the Peabody Museum of Harvard University. From Monument Valley, White Dog cave near Kay-

enta, and from the ruins near the mouth of the Chin Lee River, Arizona, numerous recoveries of seeds of *C. moschata* have been made. Specimens from Cliff House no. 1, at Monument Valley, also include the neck with the characteristic shape and white and green stripes of the cushaw. Specimen no. A3195 from the White Dog cave is ancient material. According to KIDDER and GUERNSEY, it is from the basket-makers, a culture antedating the cliff-dwellers. They are the most ancient agricultural peoples on the North American continent of whom we have any knowledge·
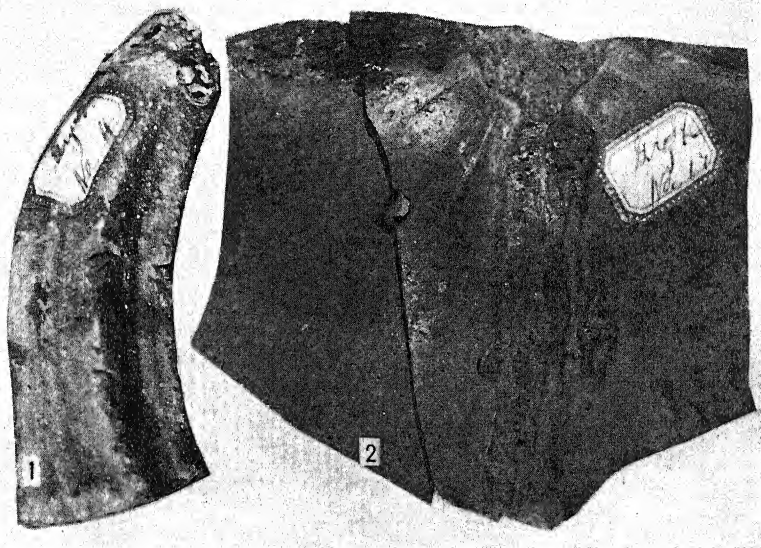
FIGS. 1, 2.—Fragments of rind and peduncle of *C. moschata* recovered by WETHERILL at Mesa Verde, Colorado (courtesy of Colorado State Historical Museum).

KIDDER states that it is possible that the basket-makers as we know them lived as long ago as 1500 or 2000 years B.C.

NEIL M. JUDD, curator of American Archeology, Smithsonian Institution, states that fragments of cucurbits recovered during explorations for the National Geographic Society at prehistoric Pueblo Bonito, New Mexico, have been identified at the Bureau of Plant Industry, United States Department of Agriculture, and confirmed by COVILLE. Among these fragments both stems and seed of *C. pepo* and *C. moschata* are present; the former predominates. In the museum of the Colorado State Historical Society are specimens collected by A. WETHERILL at Mesa Verde, which are identified by the writer as *C. pepo*. L. H. BAILEY, who has made an

extended study of the cucurbits, informs me that he has received seeds from a pottery vessel taken in Arizona that are very old, and which he identifies as *C. pepo*.

In the Field Museum of Natural History are a number of interesting vases recovered by SAFFORD from the prehistoric cemeteries near Lima, Peru. They are funerary vases modeled after pumpkins, and bearing striking resemblance in details to the summer crookneck, which is a variety of *C. pepo*.

FIG. 3.—Mortuary bowl collected by SAFFORD in prehistoric cemetery near Lima, Peru (courtesy of Field Museum of Natural History); note striking resemblance to summer crookneck pumpkin, a variety of *C. pepo*, after which this vase was apparently modeled.

It is also interesting to note that JOHN K. SMALL has collected, in the everglades of Florida, a plant of unknown origin which he identifies as *C. moschata*.[1] From southern Texas, L. H. BAILEY writes regarding *C. texana*, a form allied to *C. pepo*, which has every indication of being indigenous to that region.

The evidence, therefore, seems rather conclusive that *C. moschata* and *C. pepo* are indigenous to North America. It may also be noted that, in

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[1] Since the preparation of this manuscript, SMALL has described this plant as *Pepo (Cucurbita) okeechobeensis*, sp. nov.

the case of *C. maxima*, it is not represented in any of the archeological collections so far as I have been able to learn. Since it is a closely allied species it is not unreasonable to assume that it is also of American origin, and it is hoped that the archeologists may later have something to contribute in this case also.—A. T. ERWIN, *Iowa State College, Ames, Iowa.*

CURRENT LITERATURE

Penicillia

Mycologists are indebted to THOM for a second substantial and useful mycological and biochemical contribution. The first was the earlier volume on *Aspergilli*, and now a volume[1] on the *Penicillia* has appeared. The value of these volumes lies in the fact that they represent years of labor on the very organisms which are considered dreadful nuisances by most laboratory workers.

The volume comprises an Introduction; Characteristics of the genus (chapter I); History of *Penicillia* (chapter II); Herbaria, exsiccati, culture collections (chapter III); Generic considerations and usages (chapter IV); Culture of *Penicillia* (chapter V); Observations and descriptions of *Penicillia* (chapter VI); Physiological activities (chapter VII); Biochemistry (chapter VIII); Distribution and significance in nature and industry (chapter IX); *Penicillia* pathogenic toward man and other animals (chapter X); Classification and subdivision (chapters XI–XXV), in which it is proposed to gather the *Penicillia* into four divisions: Monoverticillata, Assymetrica, Biverticillata symmetrica, Polyverticillata symmetrica; Species of other genera described as *Penicillia* (chapter XXVI); Species identification (chapter XXVII); and Bibliography (chapter XXVIII). There is a general index and a species index.

In spite of the heroic efforts to reduce the number of species, the volume recognizes 678 species of *Penicillia*.

The volume represents an enormous labor by a most competent and experienced specialist, and goes a long way to take some of the mystery out of this genus. It is indispensable in every laboratory where microcultural work is pursued. In addition, it is a veritable *vade mecum* for specialists in fermentation, and for those dealing with the study and control of spoilage of fresh fruits and vegetables and of their products, or of animal products.—G. K. K. LINK.

A new Permian flora

A valuable contribution to our knowledge of Permian floras has been made by WHITE[2] in his description of the flora of the Hermit shale of the Grand Canyon of Arizona. The volume deals with (1) geological environment, (2) composi-

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[1] THOM, C., with assistance of CHURCH, MARGARET B., MAY, O. E., and RAINES, M. A., The *Penicillia*. pp. xiii+644. *figs. 99.* Williams and Wilkins. Baltimore. 1930.

[2] WHITE, DAVID, Flora of the Hermit shale, Grand Canyon, Arizona. pp. 221. Carnegie Inst. Washington. December, 1929.

tion and relations of the Hermit flora, (3) description of the Hermit flora, and (4) the animals from the Hermit shale.

Two interesting features are strikingly apparent in the composition of the Hermit flora. First, it is largely composed of forms previously unknown, since the flora is younger than the Lower Permian floras described from other ages of North America. Second, the flora represents an aggregate, one part of which belongs to the western European or cosmopolitan flora while the other part is connected with the Gondwana flora of Asia and the southern hemisphere. In the Hermit flora the herbaceous plants, including the pteridosperms on the one hand and the shrubby or arborescent gymnosperms on the other are rather well balanced. The entire Calamarian group is absent, probably owing to seasons of too great aridity; also the failure to find any leaves of Cordaites or *Noeggerathiopsis* is notable. Members of the Araucarian stock, like *Walchia*, *Ulmannia*, and *Voltzia* are very representative. Excellent illustrations facilitate the study of this valuable contribution.—A. C. NoÉ.

NOTES FOR STUDENTS

X-ray mutations.—As is now well known, MULLER's[3] discovery of the induction of mutations by the application of X-rays has opened a new epoch in the history of genetical research. Never has a new technique proved more rapidly prolific of instructive results.

In *Drosophila*, OLIVER[4] has shown that the total number of mutations induced is directly proportional to the dosage used, when the only factor varied is the duration of treatment. There was no indication of a threshold dosage below which mutations would not be produced, thus making it easier to believe that naturally occuring mutations may owe their origin to a similar cause. For that matter, BABCOCK and COLLINS[5] have produced evidence that the frequency of naturally occurring mutations depends upon the amount of natural radiation in the vicinity. Today the weight of evidence seems to indicate that it is not the direct play of the X-rays upon the genes that is significant; but that X-rays and other forms of radiation, ionizing the materials through which they pass, thus set free rapidly moving electrons, and that the direct impacts of these electrons upon the genes cause the changes which we call mutations.

PATTERSON,[6] by X-raying the eggs and larvae derived from flies heterozygous for certain sex-linked genes, has produced mutation which shows up in certain

[3] MULLER, H. J., Artificial transmutation of the gene. Science **66**:84–87. 1927.

[4] OLIVER, C. P., The effect of varying the duration of X-ray treatment upon the frequency of mutation. Science **71**:44–46. 1930.

[5] BABCOCK, E. B., and COLLINS, L. J., Does natural ionizing radiation control rate of mutation? Proc. Nat. Acad. Sci. **15**:623–628. 1929.

[6] PATTERSON, J. T., The production of mutations in somatic cells of *Drosophila melanogaster* by means of X-rays. Jour. Exp. Zoöl. **53**:327–372. 1929.

sections of the resulting bodies. The size of the mutant area depends upon the stage in ontogeny at which the treatment was applied. PATTERSON has also produced reverse mutations, showing that the original mutation induced by X-ray was more than a simple destruction, as some have suggested.

Time relations in the X-raying of males have been studied by HARRIS,[7] and by HANSON and HEYS.[8]

In addition to the considerable investigation of induced gene mutations that is now being carried on, MULLER and PAINTER,[9] and also DOBZHANSKY,[10] are conducting parallel genetical and cytological investigations of the redistribution of chromosome parts that is induced by X-ray. The results constitute the most striking comfirmation yet provided of the chromosome theory of heredity and the linear arrangement of genes; for translocations of sections of the various linkage groups are accompanied by corresponding translocations of visible chromosome segments. Although the genes maintain their previous sequence in the line, the visible size of the translocated segments does not correspond perfectly with the simple map distances assigned to the linkage groups from genetical results. As MULLER himself had maintained, the regions of the linkage groups near to the points of spindle fiber attachment on the chromosomes are actually longer than the maps indicate; for the frequency of crossing over is systematically reduced in these regions.

Among plants, GOODSPEED[11] had early secured results from X-raying *Nicotiana*. GAGER and BLAKESLEE[12] now report mutations of various types following the treatment of *Datura* with radium rays.

About the most instructive plant work has been done by STADLER,[13] who was

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[7] HARRIS, B. B., The effects of aging of X-rayed males upon mutation frequency in *Drosophila*. Jour. Hered. 20:299–302. 1929.

[8] HANSON, F. B., and HEYS, FLORENCE, Duration of the effects of X-rays on male germ cells in *Drosophila melanogaster*. Amer. Nat. 63:511–516. 1929.

[9] MULLER, H. J., and PAINTER, T. S., The cytological expression of changes in gene alignment produced by X-rays in *Drosophila*. Amer. Nat. 73:193–200. 1929.

———, Parallel cytology and genetics of induced translocations and deletions in *Drosophila*. Jour. Hered. 20:287–298. 1929.

[10] DOBZHANSKY, T. L., Genetical and cytological proof of translocations involving the third and the fourth chromosomes of *Drosophila melanogaster*. Biol. Zentralbl. 49:408–419. 1929.

[11] GOODSPEED, T. H., Cytological and other features of variant plants produced from X-rayed sex cells of *Nicotiana tabacum*. BOT. GAZ. 87:563–582. 1929.

[12] GAGER, C. S., and BLAKESLEE, A. F., Chromosome and gene mutations in *Datura* following exposure to radium rays. Proc. Nat. Acad. Sci. 13:75–79. 1927.

[13] STADLER, L. J., Some genetic effects of X-rays in plants. Jour. Hered. 21:3–19. 1930.

———, Genetic effects of X-rays in maize. Proc. Nat. Acad. Sci. 14:69–175. 1928.

———, Mutations in barley induced by X-rays and radium. Science 68:186–187. 1928.

independently at work along these lines while MULLER was performing his first experiments. X-raying barley seeds frequently induced gene mutation in one of the primordia of the embryo. Of the resulting tillers, one was mutant, and progenies of this one head revealed only the mutant character. Fifty-eight gene mutations were found among 2800 head progenies, as against no mutations in 1500 head progenies of the untreated control. All observed mutations were recessive; 90 per cent of them involved seedling characters, and most of these showed chlorophyll abnormalities.

Frequency of induced mutation increased in direct proportion to X-ray dosage, which was varied by changing the duration of the treatment. At the same dosage germinating seeds mutated four times as frequently as did dormant seeds, but the latter would withstand twenty times as heavy a dose without being killed.

In barley, with its seven chromosome pairs, and in those species of wheat and oats which also have seven, the induced mutation rate was about the same. Species of wheat and oats with 14 chromosome pairs, and those with 21 pairs, responded much less frequently if at all. This difference is to be expected if the species with higher chromosome numbers, having been derived originally from those with the lower, contain numerous sets of duplicate or triplicate genes. In such cases induction of single recessive gene mutations would not be detectable.

Here too X-raying also induces chromosome aberrations. Maize, in which the linkage groups are rather well known, lends itself particularly well to this study. If young developing endosperms of formula C-Sh-Wx, c-sh-wx, c-sh-wx are X-rayed, endosperm patches which are colorless, shrunken, waxy, will appear, probably through the loss of the C-Sh-Wx chromosome. The size of these patches depends upon the stage of development of the endosperm at which the X-raying occurred. Comparable effects upon the embryo are revealed by the condition of plants resulting from such grains.

STADLER feels that profitable practical application of this new technique will be limited to those situations in which hybridization is not a feasible method of plant improvement. A good example of this is the induction of bud mutations in fruit trees.

In a delightful popular article, MULLER[14] has thrown the light of all these new discoveries upon the process of evolution.—M. C. COULTER.

STADLER, L. J., The rate of induced mutation in relation to dormancy, temperature, and dosage. Anat. Rec. 41:77. 1928.

——, Chromosome number and the mutation rate in *Avena* and *Triticum*. Proc. Nat. Acad. Sci. 15:876–881. 1929.

[14] MULLER, H. J., The method of evolution. Scientific Monthly 29:481–505. 1929.

EFFECTS OF VARIATION IN DAY-LENGTH AND CLIPPING OF PLANTS ON NODULE DEVELOPMENT AND GROWTH OF SOY BEAN

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 412

SCOTT V. EATON

(WITH FIVE FIGURES)

Introduction

The importance of nitrogen fixation by legumes is universally recognized, and need not be emphasized here. In the case of most of the elements essential for the growth of plants, rocks constitute the original source. This, however, is not true of nitrogen, for the original unweathered rocks of the earth's crust are devoid of it (5). The ultimate source of the soil nitrogen is the atmosphere; here there is an inexhaustible supply. Over each acre of the earth's surface there are about 69,000,000 pounds of nitrogen (17), but, so far as we know, the ability to fix this nitrogen is possessed only by certain microorganisms, for example, certain free-living bacteria, notably *Azotobacter*, and the symbiotic or nodule-forming bacteria, various strains of *Bacillus radicicola*. Soil nitrogen, therefore, which is essential for the growth of all plants, has its ultimate source in this nitrogen-fixation process. There have been a great number of tests to determine how much nitrogen various legumes fix. While results vary with different legumes and different conditions, most of the data indicate that 100–200 pounds of nitrogen are fixed by an acre of a leguminous crop (8).

Because of the importance of nitrogen fixation by legumes, there has naturally accumulated a voluminous literature on the effects of various factors on nodule formation. No effort is made to review this literature. It is well summarized by various investigators (8, 18). Among the great number of papers on the factors affecting nodule formation, however, there are comparatively few which are concerned with those factors affecting the amount of carbohydrate manufactured by the plant in the process of photosynthesis.

LEONARD (10) found that when the amount of photosynthetic carbohydrate was decreased by growing the plants during the short days of winter, by clipping them, or by growing them in an atmosphere low in carbon dioxide, there was poorer nodule development than when photosynthesis was not decreased in any of these ways. ROSENFELS (16) grew soy beans in both poor and rich soil, exposing part of the plants of each series to a short day and part to a long day. He found that the short-day plants in each type of soil had more nodules than the corresponding long-day plants, and that the short-day, poor-soil plants had more nodules than the short-day, rich-soil plants. None of the long-day rich-soil plants developed nodules, and only 8.6 per cent of the long-day poor-soil plants. Nodule development seemed to be favored by a short day and by poor soil.[1]

The purpose of this investigation was to secure additional information on the effects on nodule development of varying the amounts of carbohydrate manufactured by the plant. It is known that root development is favored by a large supply of available carbohydrates in proportion to the amount of nitrogen present (15). It was thought that if photosynthesis should be decreased by shortening the daily exposure to light, or by clipping the plants, there might be poorer root and nodule development on account of a lack of carbohydrates in proportion to the nitrogen supply. Nitrogen fixation has usually been considered an endothermic process. As pointed out by LEON-

[1] Since this paper was written, an article by WELTON and MORRIS has appeared (WELTON, F. A., and MORRIS, V. H., Effect of fertility on the carbohydrate-nitrogen relation in the soy bean. Plant Physiol. 5:607–612. 1930). They found, as did ROSENFELS, that soy bean plants grown in poor soil developed more nodules than when grown in rich soil, and also had a higher percentage of carbohydrates but about the same percentage of nitrogen.

ARD (**10**), it is usually considered that the bacteria make use of carbohydrates manufactured by the plant in the process of photosynthesis. It was thought that by reducing photosynthesis in the ways just described a point might be reached where nodule development would fail, at least partly, on account of there not being enough carbohydrate to serve as energy and building material in this endothermic process.

Professor CHARLES A. SHULL suggested the problem to the writer. Two of Dr. SHULL's students, Dr. A. F. BARSS and Miss NAOMI MULLENDORE, studied as a class problem the effects on the growth of Golden Wax beans and of peas of brief exposures to light. The exposures varied from 1 minute to 4 hours per day. In the case of the beans there were no nodules observed on the roots except in the case of the plants exposed to light 4 hours per day, and here only one nodule of any size was found. It was thought worth while to try longer exposures. Most of the experimental work was done in the spring of 1929, but at the suggestion of Professor E. J. KRAUS, part of the work was repeated in the fall of 1929.

Methods

In all experiments plants of *Soja major* (L.) Piper were used. There were three distinct experiments. Experiments I and II were run in triplicate; experiment III in quadruplicate.

EXPERIMENT I.—This experiment consisted of the exposure of soy bean plants to daylight for periods varying in length. The following exposures were made, the figures giving the number of hours' exposure each day and the period of the day during which the exposure was made: 2 hours, 9:50 A.M.–12:00 noon; 3 hours, 9:50 A.M.–1:00 P.M.; 4 hours, 9:50 A.M.–2:00 P.M.; 5 hours, 9:00 A.M.–2:00 P.M.; 6 hours, 9:00 A.M.–3:00 P.M.; 8 hours, 8:00 A.M.–4:00 P.M.; 10 hours, 6:00 A.M.–4:00 P.M.; 12 hours, 6:00 A.M.–6:00 P.M.; full day-length, about 13.5 hours. This plan of exposure was followed except during the last few days of the experiments, when the change of time to the daylight-saving system necessitated a few slight changes as to the exposure periods. It will be noticed that in a few cases the length of the exposures does not correspond exactly with the number of hours indicated. The changes were made necessary

on account of classes, but, for convenience, throughout the paper the number of hours' exposure to light is given in whole numbers. When the plants were not being exposed to the light they were kept in a dark box in the same room in which the light exposures were made.

EXPERIMENT II.—The plants of this experiment were clipped with different degrees of severity. Those of one series were not clipped at all. These were the same plants as those of the full day-length series of experiment I. From the plants of a second group the cotyledons and first true leaves were removed. The plants of a third group were kept clipped to one leaf.

EXPERIMENT III.—Experiments I and II were tried in the spring of 1929; experiment III, in the fall of 1929. In the case of the latter experiment, one group of plants was exposed to 7 hours of natural illumination per day, the period of illumination being 8:00 A.M.–3:00 P.M. The plants of a second group were exposed to natural daylight until 4:00 P.M., at which time two 1000 watt bulbs, placed about 2 feet above the plants, were turned on. These were left on until 10:00 P.M., thus giving a day-length of about 16.3 hours.

The seeds used in all the experiments were of the variety Wisconsin Black, and were secured through the kindness of Dr. L. F. GRABER of the Department of Agronomy of the University of Wisconsin. Before planting the seeds they were inoculated with an inoculum kindly supplied by Dr. I. L. BALDWIN of the Department of Agricultural Bacteriology of the University of Wisconsin. The seeds were planted in no. 3 pure quartz sand in 2-gallon glazed earthenware crocks. Twenty pounds of sand were weighed out for each crock. In the bottom of each crock was a hole to allow for drainage. In order to get uniform plants, seeds of as nearly uniform size as possible were selected, although the individual seeds were not weighed. An excess of seeds was planted in each pot, and about 2 weeks after they had sprouted they were thinned to 6 uniform plants to the pot. In the case of a few pots, 6 uniform plants were not obtainable, so a smaller number was left. It is believed that by this plan moderately comparable material in the different series was obtained.

The nutrient solution used was a modified form of that recom-

mended by BRYAN (2). The composition of this solution, as used by BRYAN, was as follows:

Solution A: $CaCl_2$, 50 gm.
             $Ca(NO_3)_2$, 50 gm.
             Distilled water, 500 cc.
Solution B: $MgSO_4$, 50 gm.
             Distilled water, 500 cc.

Solution C: K_2HPO_4, 50 gm.
             Distilled water, 500 cc.
Solution D: $FeCl_3$, 2.5 gm.
             Distilled water, 300 cc.

Ten cc. of each of solutions A, B, C, and 10 drops of solution D were added to 4 liters of distilled water. This solution was modified by reducing the nitrate content of solution A to such a point that when diluted for use the nutrient solution contained 3 mg. of nitrate nitrogen per 100 cc. FRED and co-workers (6) found that if there was present a larger amount of nitrate nitrogen than 5 mg. per 100 cc. of nutrient solution, nodule development was partly inhibited in the case of vetch. The plants were watered daily. For the most part the watering was done with the nutrient solution, but on 2 days each week distilled water was added instead of the solution. The nutrient solution and the water were sprinkled on to the surface of the sand with a small sprinkling can. Twice each week enough of the nutrient solution was added so that it ran out through the holes in the bottom of the crocks. It was thought that this manner of adding the solution facilitated aeration, and also helped to keep the solution at a constant concentration. While the pots were not weighed, an attempt was made to keep the sand in each pot equally moist.

It was not possible to control accurately the temperature and humidity in the greenhouse, and there were considerable variations in these conditions from day to day. There may have been also, especially on bright warm days, some differences in these conditions inside the box and outside. Both in regard to temperature and humidity in the greenhouse, and differences in these conditions inside and outside the box, conditions were much more constant in the autumn than in the spring. Measurements showed that in the autumn the temperature and the humidity did not change much from day to day in the greenhouse, and that the differences inside and outside the box were small. Although these conditions were not as constant as was desired, the rather definite and regular response

of the plants, especially as regards size and color, to variations in the day-length and in the severity of clipping certainly indicated that differences in the treatment and not in temperature and humidity accounted for the results obtained.

The seeds for experiments I and II were planted March 29. The first plants were observed coming up on April 5. The plants were harvested on May 3, at which time there were a few blossoms on the plants exposed to full day-length. The seeds for experiment III were planted September 29, and the first plants were observed coming through the sand on October 7. Harvesting was done on November 6, at which time the short-day plants were in bloom and a few small pods had formed. No buds were observed on the long-day plants. Late in the evening of the day before the plants were harvested, all the plants of each experiment were put in the dark box and left until harvested next day.

SAMPLING.—The sand was carefully shaken from around the roots by jarring the crock on a board; the roots were immersed in a 20 per cent salt solution to remove the sand, and rinsed in distilled water. The plants were then wrapped in several thicknesses of newspaper and taken at once to the laboratory. Each series was harvested separately. At the laboratory the tops and roots were separated and the nodules picked from the roots. The nodules were weighed, a little 95 per cent alcohol was added, and they were later dried to constant weight in the vacuum oven at a temperature of about 80°C. The tops and roots were sampled separately. They were ground twice through a Russwin mill. About 0.25 gm. of $CaCO_3$ was added to each Erlenmeyer flask to neutralize any acids present in the tissue. The samples were weighed out, enough hot 95 per cent alcohol added to make the final percentage at least 80 per cent, and the samples heated on the steam-bath for 1 hour, small funnels being placed in the mouths of the Erlenmeyer flasks to reduce the evaporation of the alcohol. Any alcohol evaporating during the heating was replaced. The samples were stoppered tightly and stored.

METHODS OF ANALYSIS.—In the case of experiments I and II, the moisture content of each sample was determined by subtracting the sum of the dry weight of the residue left after the extraction of the sugar, and the dry weight of the total solids of the sugar extract

from the wet weight of the sample. The moisture content of the plants of experiment III was determined by taking duplicate samples of the ground pulp in tared watch glasses, which had ground edges and were held together by means of a clamp, and drying to constant weight in the vacuum oven.

The sugar extract was obtained by extracting the pulp on the steam-bath in 80 per cent alcohol for a total period of not less than 4 hours, the extract being poured off three times during this period. After the extraction, the pulp was brought on to the filter and washed with hot 80 per cent alcohol. All the extracts from each sample were made up to the mark in a volumetric flask. Tests showed that this method gave complete extraction of the sugar.

The sugar extract was freed of alcohol by evaporation on the steam-bath, cleared with neutral lead acetate, and deleaded with potassium oxalate. The reducing sugars were determined in the extract thus obtained. For the determination of the total sugars, 5 cc. of HCl was added to 50 cc. of the prepared extract; the solution was heated on the metal part of the steam-bath for 1 hour, and then set aside for 24–48 hours. The extract was neutralized with sodium carbonate and the reduction made.

The acid-hydrolyzable material was determined in the residue left after the extraction of the sugar. This residue was ground to a fine state and dried to constant weight in the vacuum oven. Hydrolysis was effected by adding to each sample 200 cc. of water and enough HCl to make a 2.5 per cent solution, and boiling for 3 hours under a reflux condenser. The sugar extract was cleared and deleaded as before and the sugar determined in this prepared extract.

In all cases the sugar of the extracts was determined by the gravimetric method, the sugar equivalent of the cuprous oxide being ascertained by reference to the Munson and Walker tables. The total sugars and the reducing sugars were calculated as invert sugar; the acid-hydrolyzable material as glucose. The percentage of sucrose was found by subtracting the percentage of the reducing sugars from the percentage of the total sugars and multiplying the result by 0.95.

Total nitrogen was determined separately in the sugar extract and the dry residue by the Kjedahl method modified to include the

nitrogen of nitrates. The method used was essentially that of RANK-
ER (14). The samples of the sugar extract were evaporated to dry-
ness on the steam-bath before making the determination.

Data

EXPERIMENT I: EFFECTS OF VARYING DAY-LENGTH IN SPRING

SIZE, COLOR, AND BUD DEVELOPMENT.—On April 29, 4 days before
harvesting, observations were made on size, color, and bud develop-
ment of all the plants. The 2-hour plants died about 3 weeks after
coming through the sand; so not many data were obtained from this
series. The plants of the other series showed a gradual decrease in
size with decrease in the length of daily exposure to light. The de-
crease in size had to do not so much with their height, although there
was some decrease here, as with size of leaflets, thickness of stems,
and general thriftiness. The plants of the shorter exposures, especial-
ly the 3-hour plants, had a rather sickly appearance. There were
brown spots on the leaves. Spots of the same kind were observed
on the leaves of the 2-hour plants, before these died, and at this time
they were more numerous on the plants of this series than on the
3-hour plants. Fig. 1 gives some idea of the appearance of the
plants of the 2-hour, 8-hour, and full day-length exposures. This
picture was taken on April 25. The 2-hour plants are practically
dead; the 8-hour plants are somewhat thrifty looking although defi-
nitely smaller than those of the full day-length series.

The plants of the full day-length, 12-hour, and 10-hour series
showed no marked differences in color, although the 10-hour plants
were somewhat less green than the full day-length and the 12-hour
plants. But from the 10-hour series to the 3-hour inclusive, there
was a gradual decrease in degree of greenness.

Well developed buds were observed on the plants of the 8-hour
and longer exposures. The plants of the 6-hour, 5-hour, and 4-hour
exposures showed definite buds, although not so well developed as
in the case of the plants of the longer exposures. No buds were ob-
served on the 3-hour plants.

The well known effect of etiolation, one characteristic of which is
lengthening of the internode, was evident. On April 20 the height of
each plant in each series was determined. The average height (in

cm.) of the plants of each series was as follows: full day-length, 8.2; 12-hour, 7.7; 10-hour, 8.0; 8-hour, 7.9; 6-hour, 9.7; 5-hour, 9.9; 4-hour, 9.4; 3-hour, 12.3; 2-hour, 15.0. The plants of the 6-hour and shorter exposures clearly show the effect, and it is especially marked in the case of the 3-hour and the 2-hour plants. On April 29 the plants of the full day-length and the 3-hour series were again measured. (The 2-hour plants were dead at this time.) The plants of the former series averaged 13.8 cm. in height; of the latter, 12.8 cm.
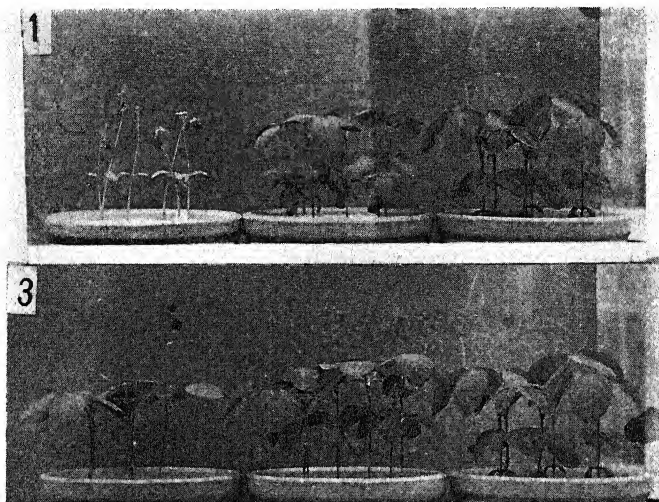
FIGS. 1, 3.—Fig. 1, effects of exposing soy bean plants to different day-lengths in spring: left, exposed to sunlight 2 hours per day; middle, 8 hours; right, full day-length (about 13.5 hours). Fig. 3, effects of clipping: left, clipped severely, only one fully developed leaf being left; middle, only cotyledons and first true leaves removed; right, unclipped.

Owing to their greater food supply, the plants exposed to full day-length increased much more in height than the plants exposed to only 3 hours of daylight each day, and more than overtook the latter, which increased very little in height.

WEIGHTS AND RESULTS OF CHEMICAL ANALYSES.—Table I shows that as the day-length decreases there is a decrease in the dry weights of the nodules, and correlated with this decrease is a decrease in the dry weights of the plants and in the amounts of total

sugars, acid-hydrolyzable material, total carbohydrates, and total
nitrogen. The table also gives the number of nodules for the plants
of each series. As is probably to be expected, these data are not so
closely correlated with the weights of the plants and the amounts
of their chemical constituents as are the weights of the nodules.
Fig. 2 gives the data in a graphical way. It will be noted that all
the curves flatten out in the parts having to do with the shorter
exposures. But, beginning with the 5-hour or 6-hour exposure, all

TABLE I

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS INCLUDED

SERIES*	DRY WEIGHT	TOTAL SUGARS	ACID HYDRO- LYZABLE MATERIAL	TOTAL CARBO- HYDRATES	TOTAL NITROGEN	DRY WEIGHT OF NODULES	No. OF NODULES
F..............	10.682	0.105	1.670	1.775	0.380	0.923	176.6
12.............	8.855	0.104	1.414	1.518	0.273	0.709	225.0
10†............	1.884	0.020	0.346	0.366	0.028	0.550	146.6
8..............	5.991	0.062	0.937	0.999	0.231	0.258	107.1
6..............	4.510	0.054	0.656	0.710	0.190	0.040	48.2
5..............	2.931	0.021	0.476	0.497	0.145	0.012	24.6
4..............	2.097	0.006	0.319	0.325	0.118	0.002	12.0
3..............	1.795	0.000	0.259	0.259	0.089	0.001	5.0

* In this and the following tables, the letters and figures of the "Series" column indicate: F=full day-length (about 13.5 hours between sunrise and sunset); FE=full day-length plus electric light (about 16.3 hours' total exposure); T=tops; R=roots; U=unclipped; CL=clipped lightly; CS=clipped severely; figures are the no. of hours' exposure to light per day.

† Data of this series are for roots only.

the curves rise rather sharply and more or less parallel with one an-
other, except the curve for the total nitrogen, which remains flatter
than the others and is not parallel with them. No data are given
for the 2-hour series, which died early. It should be stated, however,
that the roots of this series were examined, and 6 very small nodules
observed.

Table II gives the data for the tops and roots separately. It is to
be noted that, as in the case of the entire plant (table I), the data
for the tops and the roots are correlated with the length of exposure,
although the correlation is closer in the case of the tops than in the
case of the roots. The weights of the nodules (table I), therefore,
which are correlated with the length of the exposure, the dry weights,
and the amounts of the chemical constituents of the entire plant,
are more closely correlated with the dry weights and the total

amounts of the chemical constituents of the tops than with these data for the roots.

Tables III and IV give data for the percentage chemical composition of the plants. Dry weights of nodules (table I) are correlated, although not in an exact way, with the percentages of acid-hydrolyzable
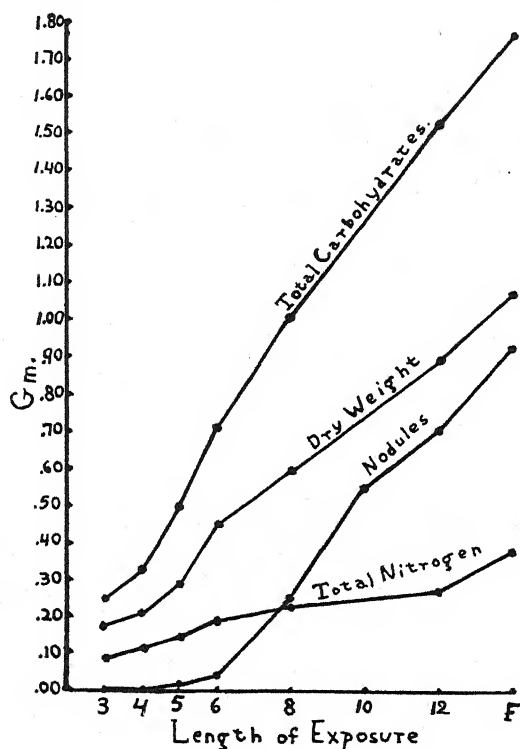
FIG. 2.—Effects of exposing soy bean plants to different day-lengths in spring; absolute gm. per 10 plants, tops and roots included: total carbohydrates, dry weight of plants (divided by 10), dry weight of nodules, and total nitrogen; figures on abscissae are number of hours' exposure to sunlight per day (F=full day-length, about 13.5 hours).

material and dry weights of the tops, but not with the corresponding percentages of the roots. Both dry weights of nodules and percentages of acid-hydrolyzable material in the tops decrease with decrease in the length of exposure to light. Percentages of dry weight, however, are even more closely correlated with weights of nodules than

are percentages of acid-hydrolyzable material. There is no correlation between percentages of total sugars in either tops or roots and weights of nodules.

TABLE II

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS SEPARATELY

SERIES	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL CARBOHYDRATES	TOTAL NITROGEN
FT........	8.533	0.086	1.297	1.383	0.339
12T........	6.509	0.076	0.983	1.059	0.233
10T........	....	....	....	....	....
8T........	4.659	0.055	0.698	0.753	0.198
6T........	3.214	0.040	0.449	0.489	0.164
5T........	2.174	0.014	0.304	0.318	0.129
4T........	1.513	0.006	0.211	0.217	0.098
3T........	1.253	0.000	0.157	0.157	0.083
FR........	2.149	0.019	0.373	0.392	0.041
12R........	2.346	0.028	0.431	0.459	0.040
10R........	1.884	0.020	0.346	0.366	0.028
8R........	1.332	0.007	0.239	0.246	0.032
6R........	1.296	0.014	0.207	0.221	0.026
5R........	0.757	0.007	0.172	0.179	0.016
4R........	0.584	0.000	0.108	0.108	0.020
3R........	0.542	0.000	0.102	0.102	0.006

TABLE III

PERCENTAGE COMPOSITION; DRY WEIGHT BASIS EXCEPT
DRY WEIGHT AND MOISTURE

SERIES	MOISTURE*	DRY WEIGHT*	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL NITROGEN
FT..........	83.862	16.138	1.022	15.200	3.976
12T........	85.873	14.127	1.179	15.200	3.588
10T........	....	....	....	....	....
8T........	86.525	13.475	1.196	15.000	4.265
6T........	86.203	13.797	1.268	14.000	5.124
5T........	89.537	10.463	0.673	14.000	5.954
4T........	90.740	9.260	0.442	14.000	6.493
3T........	91.155	8.845	0.000	12.560	6.651
FR........	90.634	9.366	0.883	17.400	1.947
12R........	89.443	10.557	1.212	18.400	1.712
10R........	90.678	9.322	1.072	18.400	1.537
8R........	90.908	9.092	0.579	18.000	2.457
6R........	78.816	21.184	1.156	16.000	2.044
5R........	84.132	15.868	1.050	22.800	2.198
4R........	87.682	12.318	0.000	18.500	3.523
3R........	84.878	15.122	0.000	18.933	1.195

* Moisture and dry weight percentages are on basis of wet weight of sample.

Percentages of moisture in the tops increase with decrease in length of exposure to light, as is probably to be expected. This is not true of the roots. It would seem that an error must account for the very low moisture content of the roots of the 6-hour exposure, which of course affects the other data for this series. The percentage nitrogen content of the tops increases with decreasing daylength. The data for the percentage nitrogen content of the roots show considerable irregularity. Sucrose was determined in only three of the series. Table IV shows that most of the total sugars of these series were made up of reducing sugars. On the average,

TABLE IV

PERCENTAGE OF SUGAR FRACTIONS; DRY WEIGHT BASIS

SERIES	TOTAL SUGARS	REDUCING SUGARS	SUCROSE
FT	1.022	0.766	0.243
12T	1.179	0.955	0.212
8T	1.196	0.892	0.288

the reducing sugars made up about three-fourths of the total and sucrose one-fourth.

The top-root ratios were determined by dividing the dry weights of the roots of 10 plants into the dry weights of the tops of 10 plants. The ratios for the different series are: Ft, 3.970; 12T, 2.774; 10T, ———; 8T, 3.497; 6T, 2.479; 5T, 2.871; 4T, 2.590; 3T, 2.311. It is to be noted that the plants of the longest exposure had the largest ratio and those of the shortest exposure the smallest, but that there is no definite gradation between these two extremes.

EXPERIMENT II: EFFECTS OF CLIPPING

Fig. 3 shows the appearance of the plants. Tables V-VIII give the data for this experiment. Table V shows strikingly the effects of clipping on nodule development. The more severely the plants were clipped the less was the weight of the nodules. The table also shows that the weights of nodules are correlated with the dry weights of the plants and with the amounts of total sugars, acid-hydrolyzable material, and total carbohydrates.

As in the case of experiment I, it is to be noted that the number of the nodules is not as closely correlated with the dry weights and

TABLE V

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS INCLUDED

SERIES*	DRY WEIGHT	TOTAL SUGARS	ACID-HYDRO-LYZABLE MATERIAL	TOTAL CARBO-HYDRATES	TOTAL NITROGEN	DRY WEIGHT OF NODULES	NO. OF NODULES
U..............	10.682	0.104	1.670	1.774	0.380	0.923	176.6
CL.............	6.828	0.054	1.061	1.115	0.241	0.645	181.2
CS.............	3.388	0.031	0.550	0.581	0.102	0.242	112.3

* U = unclipped; CL = clipped lightly; CS = clipped severely.

TABLE VI

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS SEPARATE

SERIES	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL CARBOHYDRATES	TOTAL NITROGEN
UT..........	8.533	0.086	1.297	1.383	0.339
CLT.........	4.862	0.036	0.719	0.755	0.210
CST.........	2.122	0.020	0.318	0.338	0.080
UR..........	2.149	0.018	0.373	0.391	0.041
CLR.........	1.966	0.018	0.342	0.360	0.031
CSR.........	1.266	0.011	0.232	0.243	0.022

TABLE VII

PERCENTAGE COMPOSITION; DRY WEIGHT BASIS EXCEPT DRY WEIGHT AND MOISTURE

SERIES	MOISTURE*	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL HYDROGEN
UT..........	83.862	16.138	1.022	15.200	3.976
CLT.........	85.910	14.090	0.756	14.800	4.338
CST.........	86.443	13.557	0.973	15.000	3.793
UR..........	90.634	9.366	0.883	17.400	1.947
CLR.........	90.352	9.648	0.953	17.400	1.592
CSR.........	91.461	8.539	0.938	18.400	1.788

* Moisture and dry weight percentages are on the basis of wet weight of sample.

the amounts of the chemical constituents of the plants as are the weights of the nodules. Fig. 4 records the data in the form of curves. These are almost straight lines, and also approximately parallel with

one another, except the curve for the total nitrogen, which, as in the case of experiment I, is somewhat flatter than the other curves.

Table VI shows that the dry weights of the nodules (table V) are correlated in a general way with the dry weights and the amounts of the chemical constituents of tops and roots considered separately, although more closely correlated with these data for the tops than for the roots. It should be stated that the clippings were not saved, so that the data for the tops, in the case of the clipped series, are for the part left at the time of harvest.

When the percentage composition of the plants is considered (table VII), however, it is seen that the only correlation is between weights of nodules and percentages of dry weights of the tops, there being no correlation between weights of nodules and percentages of dry weights of the roots or percentages of sugar or acid-hydrolyzable material in either tops or roots. The percentage of moisture of the tops increases directly with the severity of the clipping. This is probably to be expected, since

FIG. 4.—Effects of clipping; absolute gm. per 10 plants, tops and roots included: total carbohydrate, dry weight of plant (divided by 10), dry weight of nodules, total nitrogen. CS=clipped severely, to one fully developed leaf; CL=clipped lightly, only cotyledons and first true leaves removed; UC=unclipped.

the more severely the plants are clipped the more transpiring surface is removed. The moisture percentages of the roots bear no direct relation to the severity of the clipping. The percentages of total nitrogen do not vary directly with the severity of the

clipping in either tops or roots. Table VIII shows that sucrose makes up a greater percentage of the total sugars in the case of the plants which were clipped lightly than in the case of the plants which were not clipped. This may be due at least partly to the fact that the clippings were not saved.

TABLE VIII

PERCENTAGES OF SUGAR FRACTIONS; DRY WEIGHT BASIS

SERIES	TOTAL SUGARS	REDUCING SUGARS	SUCROSE
UT.........	1.022	0.766	0.243
CLT........	0.756	0.499	0.244

The top-root ratios were determined. The ratios for the three series are: FT, 3.970; CLT, 2.473; CST, 1.676. Since the clippings were not saved these ratios are probably not significant.

EXPERIMENT III: EFFECTS OF VARIATION IN DAY-LENGTH
IN FALL; NATURAL DAYLIGHT SUPPLEMENTED
WITH ELECTRIC LIGHT

Fig. 5 gives some idea of the differences in appearance of the long- and the short-day plants. The picture was taken a few days before the plants were harvested. The long-day plants were much taller than the short-day plants and were twining about the supports, while the short-day plants were not twining. On November 2, 4 days before the plants were harvested, the longest plant in each pot was measured and observations made of all the plants as to color, occurrence of hairs, and bud development. The average height of the long plants in the long-day series was 43.7 cm.; in the short-day series, 22.5 cm. The long-day plants were much darker green than the short-day plants and showed a much greater development of hairs. There were a number of buds on each of the short-day plants and a few had opened. No buds were observed on the long-day plants. When the plants were harvested, 4 days later, a few small pods were observed on the short-day plants.

The weights of the plants and the results of the chemical analyses are found in tables IX-XII inclusive. By reference to table IX, it

is seen that the weight of the nodules of the long-day plants is much greater than that of the short-day plants, and that correlated with this greater weight of nodules is a greater dry weight and greater amount of total sugars, acid-hydrolyzable material, total carbohydrates, and total nitrogen in the case of the long-day plants as compared with the short-day plants. The weights of the nodules are on

FIG. 5.—Effects of exposing soy bean plants to different day-lengths in autumn: left, exposed to sunlight 7 hours per day; right, exposed to sunlight from sunrise to 4:00 P.M. and to electric light from then to 10:00 P.M., giving total exposure of about 16.3 hours.

the wet weight basis. Since the nodules probably have about the same percentage of moisture as the rest of the plant, however, this fact does not greatly vitiate the comparison of weights of nodules and dry weights of plants. Table X shows that this same comparison holds for the tops and roots considered separately, although more closely for the tops than for the roots.

Table XI gives the percentage composition of the plants; table XII gives the percentages of the sugar fractions. Weights of nodules

(table IX) are correlated in a general way with percentages of acid-hydrolyzable material of tops and roots, although the correlation is

TABLE IX

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS INCLUDED

SERIES	DRY WEIGHT	TOTAL SUGARS	ACID-HYDRO-LYZABLE MATERIAL	TOTAL CARBO-HYDRATES	TOTAL NITROGEN	WET WEIGHT OF NODULES	No. OF NODULES
FE............	13.477	0.375	2.708	3.083	0.478	4.136	167.3
7.............	4.917	0.107	0.790	0.897	0.208	0.663	86.3

TABLE X

ABSOLUTE GM. PER 10 PLANTS; TOPS AND ROOTS SEPARATE

SERIES	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL CARBOHYDRATES	TOTAL NITROGEN
FET........	11.409	0.335	2.384	2.719	0.441
7T.........	3.889	0.080	0.645	0.725	0.196
FER.......	2.068	0.040	0.324	0.364	0.037
7R.........	1.028	0.027	0.145	0.172	0.012

TABLE XI

PERCENTAGE COMPOSITION; DRY WEIGHT BASIS EXCEPT
DRY WEIGHT AND MOISTURE

SERIES	MOISTURE	DRY WEIGHT	TOTAL SUGARS	ACID-HYDROLYZABLE MATERIAL	TOTAL NITROGEN
FET........	84.958	15.042	2.937	20.900	3.874
7T.........	87.197	12.803	2.077	16.600	5.044
FER.......	86.641	13.359	1.944	15.700	1.824
7R.........	86.814	13.186	2.650	14.200	1.203

TABLE XII

PERCENTAGE OF SUGAR FRACTIONS; DRY WEIGHT BASIS

SERIES	TOTAL SUGARS	REDUCING SUGARS	SUCROSE
FET........	2.937	2.325	0.581
7T..........	2.077	1.288	0.749

closer for the tops than for the roots. The greater weight of nodules of the long-day plants is also correlated with a greater percentage

of total sugars of the tops of these plants. This comparison, however, does not hold for the roots. Weights of nodules are correlated with percentages of dry weights of tops and roots, although the correlation is closer for the tops than for the roots.

Both the tops and the roots of the short-day plants have a higher percentage of moisture than the long-day plants, although the percentage is not much greater in the case of the roots. The percentage of total nitrogen is higher in the case of the short-day tops, but the reverse holds for the roots. The top-root ratios were determined. The ratio for the long-day plants was 5.516 and for the short-day plants, 3.783. The ratio of the long-day plants was the highest obtained in any of the experiments, while the ratio of the short-day plants is of the same order as the ratios of experiment I.

Discussion

EFFECTS ON NODULE DEVELOPMENT OF VARIATIONS IN FACTORS AFFECTING AMOUNTS OF CARBOHYDRATE MANUFACTURED

Two aspects of the relation of the chemical composition of the plant to its development should be mentioned in this connection. One is the relation of carbohydrates and nitrogen to the root development; a second is the need of the legume for carbohydrates in the process of nitrogen fixation.

Various investigators have found that a large carbohydrate supply in proportion to the amount of nitrogen present favored root development, while the reverse situation favored shoot development. For example REID (15), working with tomato cuttings, found that there was better root development when the cuttings contained a large supply of readily available carbohydrates in proportion to the amount of nitrogen present, but that there was poor root development when the cuttings contained a low carbohydrate supply in proportion to the amount of nitrogen present. It might be expected that with a decrease in day-length, or because of clipping, the percentage of carbohydrates in the plant (roots, tops, or both) would be less, and that this low carbohydrate supply in proportion to the nitrogen would result in decreased root development, and also, since the nodules may be considered a part of the root system, in decreased nodule development.

A second aspect of the question is the need of carbohydrates for the process of nitrogen fixation. This process has usually been considered an endothermic one, and it is thought that complex organic compounds, perhaps protein in nature, are synthesized. It might perhaps be expected, therefore, that when carbohydrate manufacture is reduced there would be poorer nodule development, owing to there not being sufficient carbohydrates to serve as energy and building material for this endothermic process. LEONARD (10) found that when photosynthesis by soy bean plants was decreased, owing to insufficient light, CO_2, or chlorophyll, there was poorer nodule development than in the case of plants suffering no reduction in the amount of carbohydrates manufactured. This was explained by assuming that there was not sufficient carbohydrates for the activities of the nodule-forming bacteria after the needs of the plant were supplied. ROSENFELS (16) found that short-day plants grown in poor soil developed many more nodules than long-day plants grown in the same type of soil, and that correlated with this better nodule development was a higher starch content. It was stated that this is to be expected because of the need of carbohydrates for the synthesis of the higher organic complexes. However, this correlation between nodule development and starch content was not found in the case of short-day and long-day, rich-soil plants.

In view of these two considerations, the relation of carbohydrates and nitrogen to root development, and the need of carbohydrates for nitrogen fixation, it becomes of interest to inquire as to what correlation was found between the chemical composition and the growth and nodule development of the plant as a result of variations in day-length and clipping. As shown by tables III and XI, the main effect of variations in day-length on the chemical composition of the plant was that as the day-length decreased the percentage of acid-hydrolyzable material of the tops decreased and the percentage of nitrogen increased. In the case of experiment III, table XI (but not of experiment I) this was also true, although to a lesser extent, of the roots; also the percentage of total sugars of the tops of the short-day plants of this experiment was less than that of the long-day plants. In other words, the short-day plants of experiments I and III had a smaller percentage of carbohydrates and a

higher percentage of nitrogen than the corresponding long-day
plants. This is the situation usually prevailing in the case of more
vegetative, less fruitful plants, which also have usually a smaller
developments of roots in proportion to the tops. But the short-day
plants of each experiment were less vegetative, and had a greater
development of roots in proportion to the tops than the long-day
plants. Also the short-day plants in each case were fruitful, although
in the case of experiment I the long-day plants blossomed first.
With a decrease in day-length there was poorer nodule but better
root development. As for the effects of clipping, although the
weights of nodules varied directly with its severity, table VII shows
that the percentages of the various chemical constituents did not
vary in any regular manner with the degree of clipping. This may
be due at least partly to the fact that the clippings were not saved.
The results do not seem to show a very close correlation between the
carbohydrate-nitrogen relationship of the plant and growth and
nodule development.

When the data are considered from the standpoint of the need
of carbohydrates for the process of nitrogen fixation, percentages of
carbohydrates of the plant seem to be more closely correlated with
nodule development than was noted for the carbohydrate-nitrogen
relationship of the plant. The long-day plants of experiments I and
III had better nodule development than the plants exposed to sun-
light a shorter length of time per day (tables I and IX). Also, as
noted in the preceding paragraph, these long-day plants were higher
in carbohydrates, especially acid-hydrolyzable. material. But the
correlation is far from an exact one. In the case of experiment I both
weight of nodules and percentage of acid-hydrolyzable material de-
creased with decrease in the length of exposure to light, but tables
I and III show cases of decided decrease in weights of nodules with-
out any decrease in the percentages of acid-hydrolyzable material.
Yet one would seem justified to conclude that one reason for the
decrease in nodule development as the day-length is shortened is the
decreasing supply of carbohydrates for the use of the nodule-forming
bacteria in the nitrogen fixation process. In the case of experiment
II (effects of clipping), although the weight of nodules varied direct-
ly with the severity of the clipping, table VII shows that the per-

centages of carbohydrates varied in no regular way with this severity. This may have been due to the fact that the clippings were not saved, as previously mentioned. It must be kept in mind, however, that certain recent work minimizes somewhat the need of carbohydrates for the nitrogen fixation process. It is known that the synthesis of ammonia from the elements composing it is an exothermic process (5), and, while the initial compound formed in the fixation of nitrogen by the nodule-forming bacteria is not known, there is evidence, according to Burk (1), that *Azotobacter* fixes the nitrogen in the form of ammonia. If proteins are the final products of the fixation process, however, it would seem that the entire process must be endothermic, since the proteins have a higher heat of combustion than the carbohydrates, which serve as part of the building material for the proteins. Yet Burk states that it is practically certain that energy is not essential for the synthesis of complex nitrogen compounds from ammonia. Of course, even if the steps from ammonia on are endothermic in character, the energy for these steps could come from the exothermic fixation of ammonia. In this case the entire process, including the synthesis of the proteins, might require little if any energy. From experimental results, Christiansen-Weniger (3) concluded that fixation of nitrogen by the nodule-forming bacteria is an exothermic process, and that the bacteria are able to use this energy in their life processes. This viewpoint lessens somewhat the importance of carbohydrates in the nitrogen-fixation process. However, even if they are not needed as an energy source for the fixation of nitrogen, they are needed as a carbon source for the synthesis of the complex nitrogen compounds, and any deficiency in carbohydrates would be expected for this reason to result in poorer nodule development.

Although, as just stated, nodule development is correlated at least in a general way with percentage of carbohydrates, especially acid-hydrolyzable material, a closer correlation is noted when the data are examined from another standpoint. Tables I, II, V, VI, IX, and X give on the absolute rather than the percentage basis the dry weights and the amounts of the chemical constituents of the plants. As the day-length decreases, or as the plants are clipped more severely, the weights of nodules decrease, and correlated with poorer nodule de-

velopment there is less dry weight and lesser amounts of total sugars, acid-hydrolyzable material, and total nitrogen. In other words, the nodules seem to develop as the rest of the plant develops. This is perhaps to be expected since the nodules are an integral part of the plant. This correlation is closer for the tops than for the roots (tables II, VI, X). It is also striking when the data for the entire plant are considered (tables I, V, IX, and figs. 1, 4). The figures show that the curves for the weights of nodules are more nearly parallel with those for total carbohydrates than with those for total nitrogen. So, as photosynthesis is reduced there is less growth of all parts of the plants, including the nodules. According to this viewpoint, the development of tops, roots, and nodules may be better explained by a consideration of the total amounts of the chemical constituents than of the amounts figured on a percentage basis.

This idea, that development of the plant may be more closely correlated with the chemical composition figured on an absolute rather than a relative or percentage basis, is really not in conflict, at least in the case of experiments I and II, with the viewpoint that the type of plant resulting from any treatment is determined by the chemical composition figured on the latter rather than the former basis; for in these experiments, the types of plant in the various series were similar, although there were big differences in their sizes. Bud development of the plants of the various series of experiment I was similar (except the 3-hour plants, which developed no buds), although the first buds to open were on the plants of the full day-length series. The top-root ratios of experiment I were somewhat different, but the differences were not consistent from series to series. There was a big decrease in the top-root ratios of experiment II as the severity of the clipping increased, but this is due at least in part to the fact that the clippings were not saved. If we consider the type of plant as similar in the various series of experiments I and II, therefore, the amounts of the chemical constituents figured on an absolute basis might perhaps be considered more important in explaining the differences in weights of nodules, tops, and roots, than the amounts figured on a percentage basis. The total amount of the chemical constituents present determines the amount of growth. As photosynthesis is decreased there is less growth of all the

parts of the plant, because there is less food material available. The plant is smaller, but this does not necessarily mean that there is a difference in the percentage of the chemical constituents present, at least of a magnitude and character sufficient to account for the difference in the development of the plant. According to this viewpoint the type of plant is still determined by the chemical composition figured on a percentage rather than an absolute basis, but with the type of plant in the various series similar, the chemical composition figured on an absolute basis might be regarded as more important in explaining the differences resulting from the treatments. The long- and short-day plants of experiment III are very different in type, but even in this case the amounts of the chemical constituents figured on an absolute rather than a percentage basis seem more important in explaining the development of the plant, including nodule development.

It is realized that there is need of greater fractionation of the carbohydrates and the nitrogen in any interpretation of the relation of the chemical constituents to the development of the plant. Since the work of KRAUS and KRAYBILL (9), great emphasis has rightly been placed on the importance of the carbohydrate-nitrogen relationship in the development of the plant. KRAUS and KRAYBILL regarded the nitrate-nitrogen fraction as the most important nitrogen fraction. Recent work, however, especially that of NIGHTINGALE (11), emphasizes the importance of the soluble organic nitrogen. NIGHTINGALE finds that the balance between the available carbohydrates and the soluble organic nitrogen determines the type of growth of the plant. It is unfortunate that the acid-hydrolyzable material was not separated into the hemicellulose and the starch-dextrin fractions. The functions of the hemicelluloses in the plant are not definitely known. NIGHTINGALE regards the starches and dextrins as more intimately connected with the plant's metabolism than the hemicelluloses. His work indicates that, although the hemicelluloses may be utilized by the plant as a sugar source, this does not occur until the starches and dextrins become deficient. He admits, however, that his data are insufficient for definite conclusions on this point. CLEMENTS (4), on the other hand, finds that when growing conditions are favorable, large and frequent variations in

the hemicellulose content of leaves occur, the hemicelluloses apparently serving as temporary food reserves. If the total nitrogen had been separated into the various fractions and a greater fractionation of the carbohydrates made, a greater correlation might have been found between growth and nodule development of the plant and the amounts of the chemical constituents present, figured on a percentage rather than an absolute basis, although it might still be a problem to explain the fact that nodule development was poorer in the short exposures, while root development in proportion to the tops was better.

Throughout this work the tops showed much greater response to the variations in day-length and degree of clipping than did the roots. Also the differences in the chemical composition of plants as a result of the different treatments were greater and more regular for the tops than for the roots, no matter whether figured on a percentage or an absolute basis. In one of NIGHTINGALE'S experiments it was found that long-day and short-day tomato plants had similar root systems, although the tops were very different. Also there was little difference in the relationship of the carbohydrates to the nitrogen in the roots of the long-day plants as compared with the roots of the short-day plants, although there was a decided difference in this respect in the stems of the plants of the two series. Variations in conditions, which may cause decided differences in the development of the tops of plants, may not affect the development of the roots so markedly.

EXPERIMENT III: PHOTOPERIODISM AND NODULE DEVELOPMENT

It is interesting to note that long- and short-day plants grown in the fall developed differently from the corresponding plants grown in the spring. It has been stated that when the day-length in the spring was varied the resulting plants did not vary much in type, although with decrease in day-length the resulting plants of course were smaller and less green. On the other hand, the long- and short-day plants grown in the fall were very different in type, the short-day plants being relatively non-vegetative, fruitful, and non-twining, while the long-day plants were relatively vegetative, non-fruitful, and twining (fig. 5). In regard to nodule development, however,

the plants grown in the fall were similar to those grown in the spring. In each case the long-day plants developed a much greater weight of nodules than the short-day plants (tables I and IX).

Regarding percentage composition, the long-day and the short-day plants grown in the fall were also similar to those grown in the spring. In each case the long-day plants were higher in carbohydrates, especially acid-hydrolyzable material, and lower in total nitrogen than the short-day plants (tables III and XI).

In the discussion of experiment I, it was stated that there was not a very close correlation between nodule development and the percentage composition of the plant, although from the standpoint of the need of carbohydrates for nitrogen fixation the better nodule development of the long-day plants might be regarded as being at least partly explained by their higher carbohydrate content. This same situation holds in the case of experiment III (variations of day-length in the fall). Because of the need of carbohydrates for nitrogen fixation, the higher carbohydrate content of the long-day plants might be regarded as explaining at least partly the better nodule development of these plants. But the other aspect of the relation of chemical composition to nodule development, discussed in connection with experiment I, that of the relationship of carbohydrates and nitrogen to root development, does not apply in experiment III any more than it did in experiment I. In each case the percentage of carbohydrates is less and the percentage of nitrogen is greater (this applies especially for the tops) in the short-day than in the long-day plants. This is the situation usually found in the case of highly vegetative, non-fruitful plants, which usually also have a smaller development of the roots in proportion to the tops. But, as has already been stated, both the short-day plants grown in the fall and the corresponding plants grown in the spring were less vegetative and had better development of roots in proportion to tops than the long-day plants. So neither the type of the plant nor nodule development, at least in so far as this is related to the carbohydrate-nitrogen relationship, seems to be explained by the percentage composition of the plant. Both seem to be related more closely to the amounts figured on an absolute basis. But there is need of greater fractionation of the carbohydrates and nitrogen. If

this had been done, there perhaps would have been found a closer correlation between the type of the plant and its percentage composition. However, since the roots of the short-day plants were, relative to the tops, better developed than those of the long-day plants, the carbohydrate-nitrogen relationship would still not explain the better nodule development of the long-day plants, unless we consider nodule development to be more in accord with the development of the tops than of the roots.

Others have noted similar differences in the type of soy bean plant when day-length was varied in the fall or winter (11, 16). Rosenfels found that the long-day plants were highly vegetative and non-fruitful as compared with short-day plants, and were lower in carbohydrates in proportion to the nitrogen than the short-day plants. This is the situation usually found in this type of plant. Nightingale also found that long-day soy bean plants were vegetative and non-fruitful as compared with short-day plants. The short-day plants were higher in total nitrogen, nitrate nitrogen, total sugars, and starch and dextrin, but somewhat lower in hemicellulose than the long-day plants. Nightingale did not examine the nodules on the roots; Rosenfels did, and found much better nodule development on the root of the short-day plants. This is directly opposite to my results. It would seem that further work needs to be done on the nodule development of long-day and short-day soy bean plants. Rosenfels also found that the short-day plants were higher in carbohydrates, especially starch and dextrin, in proportion to the nitrogen, than the long-day plants. He considered that this was in accord with the better nodule development of these plants.

It is rather difficult to explain the phenomenon that long-day and short-day plants grown in the fall or winter were different in type from the corresponding plants grown in the spring or summer. One factor which should perhaps be considered is the difference in the length of day of the long-day plants in the two cases. The long-day plants in the spring were exposed to light about 13.5 hours per day, in the autumn about 16.3 hours. Although Garner and Allard (7) have shown that certain varieties of soy beans are short-day plants, and continue vegetative if the day is kept too long, the difference in

day-length in the spring and autumn does not seem sufficient to account for the differences in the type of plant. The intensity of the light was of course less in the autumn than in the spring. But while, as shown by POPP (13), reducing light intensity causes twining of the soy bean, he, and also GARNER and ALLARD, found that reduction in light intensity had little if any effect on the time of flowering. There remains to be considered the quality of light. The quality of sunlight was different of course in the autumn from that in the spring; and since the long-day was secured by supplementing natural daylight with electric light, we have also to take into account the differences in the quality of electric light as compared with sunlight. Some work has been done showing that the quality of the light in which the plant is grown is very important. For example, POPP (12) grew soy beans and other plants in light of various qualities. Among the many effects noted when all rays shorter than 529 mμ were absent was a delay in the time of flowering, and also a reduction in the number of flowers produced. There is need of more work on the effects of light of various qualities on the development of plants. It seems likely that a difference in the quality of light was at least one important factor causing the plants grown in the autumn to be different in type from the plants grown in the spring. However, whatever was the external factor causing the markedly different type of plant when day-length was varied in the autumn, the carbohydrate-nitrogen relationship of the plants was not in accord with this difference in type.

Summary

1. The effects on growth and nodule development of the soy bean of varying certain factors that affect the amount of carbohydrate manufactured by the plants in the process of photosynthesis were studied. The factors varied were length of the exposure to light and the amount of photosynthetic tissue present, differences in the latter being secured by clipping the plants with different degrees of severity.

2. The amount of growth and nodule development was in direct proportion to the length of day and the degree of severity of the clipping.

3. Nodule development was correlated with percentage of carbo-

hydrates, especially acid-hydrolyzable material, of the plants. This correlation was much greater for the tops than for the roots. It is suggested that part of the decrease in nodule development resulting from decrease in photosynthesis is due to a lack of carbohydrates for the nitrogen fixation process.

4. No correlation was noted between the carbohydrate-nitrogen relationship of the plant and growth and nodule development in regard to the effect of this relationship on the development of the roots in proportion to the tops. The roots relative to the tops were better developed in the shorter than in the longer exposures. This lack of correlation may have been due to the fact that there was not sufficient fractionation of the carbohydrates and nitrogen.

5. Growth and nodule development of the plant were more closely correlated with the amounts of the chemical constituents when figured on an absolute basis than on a percentage basis. It is suggested that, with a decrease in photosynthesis there may be less growth of all parts of the plants, including the nodules, because of the smaller amount of food material present; but that the percentage composition of the resulting plants may be similar, or at least not different enough to account for the difference in the size of the plants. According to this viewpoint, the amounts of the chemical constituents figured on an absolute rather than a percentage basis may be more important in explaining the effects of the treatments.

6. The degree of greenness of the plants was in direct proportion to the length of exposure to light, and the phenomenon of etiolation was very prominent in the case of plants of the shorter exposures.

7. The plants resulting from a variation in day-length in the spring and from clipping were similar in type in regard to time of bud development, absence of twining, etc. The plants exposed to different day-lengths in the autumn were very different in type, the long-day plants being relatively vegetative, with a better development of tops in proportion to the roots, non-fruitful, and twining; the short-day plants being relatively non-vegetative, with a poorer development of tops in proportion to the roots, fruitful, and non-twining. However, nodule development of the autumn-grown plants was similar to that of the spring-grown, varying directly with the length of exposure to light.

8. Various external factors which might account for the fact that the plants exposed to different day-lengths were similar in the spring but different in the autumn are day-length, intensity of light, quality of light. Differences in the quality of the light may be an important factor.

9. Whatever were the external factors accounting for the differences in the types of plants when day-length was varied in the autumn, the carbohydrate-nitrogen relationship did not explain the differences. This may have been due to the fact that there was not sufficient fractionation of the carbohydrates and nitrogen.

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FACTORS INFLUENCING FRUIT SETTING IN THE PECAN[1]

Guy W. Adriance

(WITH SIX FIGURES)

Introduction

This study was undertaken to gain information on the effects of some cultural practices affecting fruit setting in the pecan. Since pollination is essential to complete fruit development in many flowering plants, it has been studied in association with the morphology and cytology of the developing nut.

Materials and methods

Morphology and cytology.—For the study of the morphology and cytology of the developing pecan nut, collections of material for imbedding were made at frequent intervals during the early growing season. The first samples were taken from swelling buds, and the next from tips of growing shoots, before the pistillate flowers actually appeared. Following the appearance of the pistillate flowers, collections were made each day up to the time of receptivity and pollination; after which samples were taken at 4-hour intervals during the first day after pollination, then daily for 3 days, and then at 2-day intervals for 6 weeks. During the same period, samples which had been sacked and not pollinated were taken. In the later stages the shell of the nut was too hard for sectioning, so nuts collected after 4 weeks were trimmed with a knife to remove the hard shell. All material was killed and fixed in medium chromo-acetic solution, and sectioned according to the paraffin method. The most satisfactory stains used were Delafield's haematoxylin, safranin-gentian violet, and acid fuchsin. The latter was satisfactory for staining pollen tubes.

Some sections of very large fresh fruits were cut with a knife, killed in alcohol, cleared in acetone and cedar oil, and photographed with indirect light in thin glass cells filled with Canada balsam.

FRUIT DROPPING.—Field studies were undertaken with the idea of determining the amount of shedding of young pecan fruits, the time of shedding, and the factors influencing this condition. Preliminary observations and the work of other investigators indicated that the greater portion of the drop occurs early in the season. Records were taken of the dropping of the fruit during the period up to 6 weeks after time of pollination, on open-pollinated fruits and on fruits which were kept sacked until after all pollen was shed. Some further counts were made later in the season to check the relative importance of the early drop. The position of the dropped nuts on the peduncle was determined by the scar. These determinations were made twice a year, the proximal nut being counted as number "1" in all cases.

BLOSSOMING HABIT.—The first and last dates on which flowers were receptive was recorded for each tree. The period of receptivity was determined by the presence of a very perceptible viscous fluid on the stigmatic surface. The first and last dates on which pollen was shed were also recorded for each tree. The trees used for these records are in two orchards. The "old" orchard contains trees which were about 15 years old at the beginning of the study, and had been bearing moderately good crops. Seven varieties were used in this orchard, and from 3 to 12 trees of a variety, depending on the number available. In the "young" orchard the trees were 6 years old and beginning to bear well when the first data were taken in 1927. There were 10 varieties available in this orchard, and 8 or more trees of each variety.

METEOROLOGICAL DATA.—The data on temperature and precipitation were secured from the records of the Main Station Farm, Texas Experiment Station, which is about one-quarter of a mile from each orchard. Since, according to WOODROOF (35), SHUHART (30), and ISBELL (10), the staminate flowers are differentiated in the spring of the previous year and the pistillate flowers in the same spring in which they appear, it was not considered necessary to make records except for the late winter and spring, beginning January 1, of each

season, so as to give a sufficient margin before the beginning of growth.

The temperature records for the periods under consideration for each year have been converted into heat units, in order to have a definite basis for comparison. The remainder system of calculating the number of heat units above 40° F. was considered adequate for this study; the yearly total from January 1 to time of maturity of staminate and pistillate flowers was determined for each variety. The weekly, monthly, and seasonal distribution of heat units and precipitation for each spring season were also calculated so that the effect of these factors on dichogamy might be determined.

The work is concerned with the functioning of the plant over a period of years. The seasonal study is based upon a 5-year period, and an inspection of the meteorological records used shows that extremes of temperature and rainfall were encountered during this time.

Structure of flowers and fruits

The morphology of the species of *Carya* has been treated by BENSON and WELSFORD (3), NAWASCHIN (25), VAN TIEGHEM (33), ROWLEE and HASTINGS (28), DE CANDOLLE (7), MEEHAN (16), LUBBOCK (14), BRAUN (6), NICOLOFF (26), and others. BILLINGS (4) and WOODROOF (37) worked with the pecan specifically.

FLOWERING HABIT.—With regard to the production of staminate flowers, WOODROOF (35) and ISBELL (10) state that the catkins are formed within the bud in early spring, the process probably extending through April and May. In any case the staminate flowers are differentiated early in the growing season a full year previous to their appearance, and there is no chance for a deficiency of pollen due to retarded differentiation of stamens. In 1928 a Burkett tree at College Station had two clusters of pistillate flowers sacked after all catkins around the basal portion had been removed. When the sacks were later removed, it was noticed that one lateral bud on each of the new shoots had produced a group of catkins. Since these catkins were on current season's growth, they had certainly been differentiated just previous to their appearance. The catkins appeared normal but were too late to be of any value in producing pollen for the current season. The stigmas had all dried several weeks previously, while the catkins were still green and immature.

WOODROOF and WOODROOF (36) state that the pistillate flowers are normally differentiated from the terminal buds of the previous season but may be produced from lateral buds. SHUHART (30) gives three cases of false terminal buds which produced pistillate flowers, and maintains that the true terminal bud is strictly vegetative. It has been observed at College Station that few true terminal buds are produced, and the greater part of the pistillate flowers arise from lateral buds near the apical end of the shoot. Buds rendered subterminal in position by cutting back the dormant shoot have in practically all cases produced pistillate flowers.

Detailed study of the structure of the nut in various stages of development showed the following facts:

1. The middle septum of the nut is parallel to the axis of the inflorescence, and the plane of the stigmas is also parallel to this axis. In the walnut, although the plane is parallel to the axis, as in the pecan, the middle septum is perpendicular to this axis.

2. The peculiar branching of the vascular system of the walnut, as described by NICOLOFF (26) but not previously figured for the pecan, is clearly shown in this species (figs. 1, 2). NICOLOFF states that anterior and posterior to the ovule the placenta produces two bodies, in the form of horns, which are not an integument as has been represented. The ovule, exactly terminal, according to NICOLOFF, is supplied with symmetrical vascular connections from both sides by vessels (and also phloem) which come from the lateral bundles of the transverse partition, running to a point just below the ovule, and turning back to enter the integument. The development and the anatomy of the ovule, in his opinion, show that this organ has an axil dependence and not a carpellary dependence (cauline instead of foliar).

3. A vertical partition (middle septum) supports the orthotropous, sessile ovule, which has only one integument (figs. 1, 2). After fertilization, the cotyledons expand downward on either side of the partition, away from the micropyle. In the walnut (26) the ovary, at first unilocular, becomes at the time of fertilization quadrilocular in its basal portion, and also in its upper portion; this tendency is observed to a slight extent in the basal portion of the pecan.

4. The normal bearing habit of the pecan at College Station is the production from a single compound bud, lateral and usually

subterminal, which separates as three buds when the heavy outer
bud scales are pushed off in the spring. From the two outer buds,
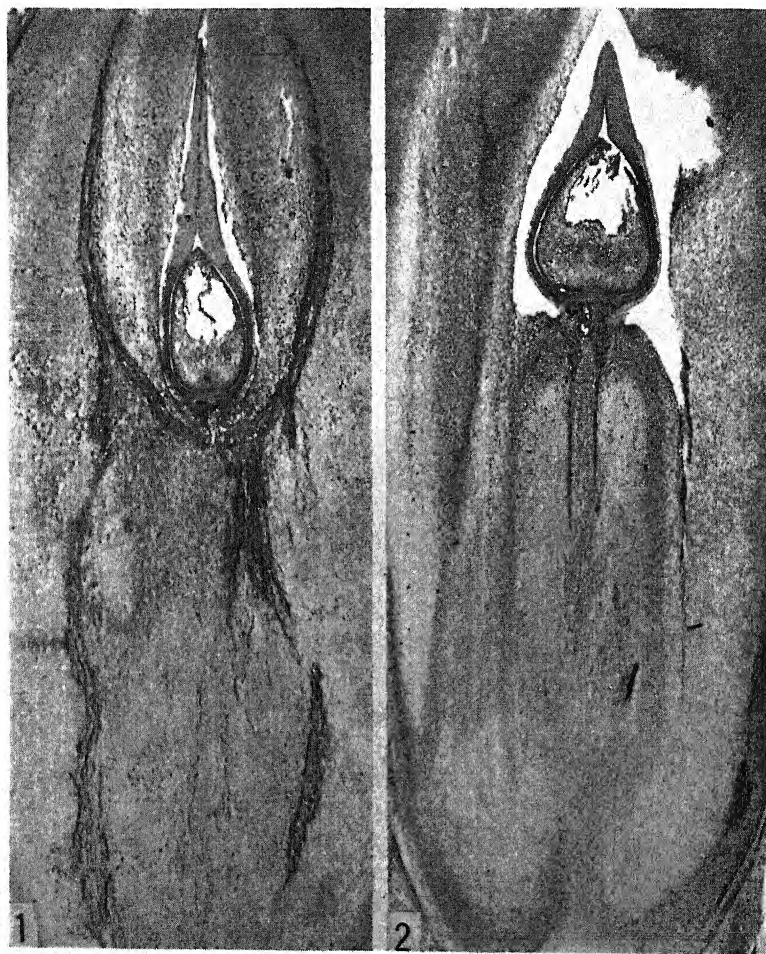
FIGS. 1, 2.—Fig. 1, longitudinal view of nut in plane of septum 6 weeks after pollina-
tion, showing gross morphology and portion of vascular connection of ovule; fig. 2,
longitudinal view of nut perpendicular to septum, 6 weeks after pollination; ×18.

two clusters of three catkins each arise with a central shoot, coming
from the bud between these two clusters, which terminates in a pis-
tillate inflorescence. This is not in harmony with the common con-

ception that the staminate flowers arise from lateral buds and the
pistillate flowers from terminal buds (fig. 3).
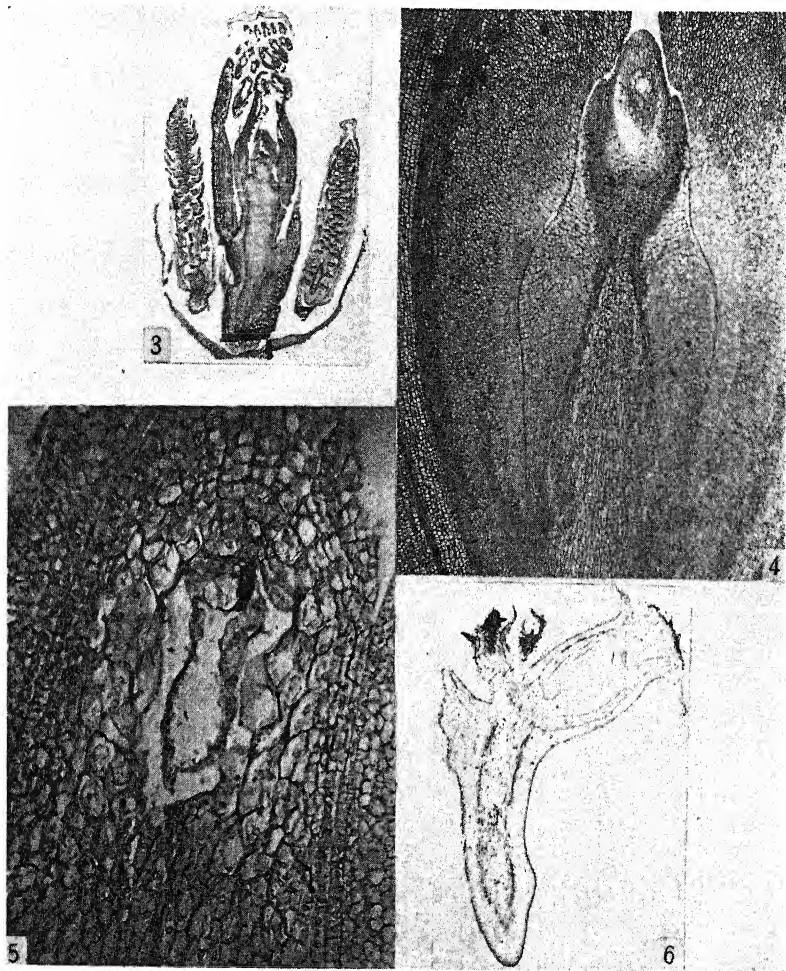
FIGS. 3–6.—Fig. 3, lateral bud of pecan at time of swelling in March, showing two
clusters of catkins and central shoot with pistillate primordia, ×18; fig. 4, ovule at time
of pollination, showing integument not yet inclosing nucellus, ×45; fig. 5, enlarged
view of ovule 3 weeks after pollination, showing fertilized egg and disintegrated syner-
gids, ×198; fig. 6, termination of pistillate flower cluster, showing one normal and two
abortive flowers, which will shrivel and drop about time of pollination, ×18.

5. In the case of the Stuart pecan, small anther-like structures were observed on the flower axis at the bases of individual pistillate flowers. This condition was observed in two clusters, and no previous record of such structure has been found.

Pollination and fertilization

Chalazogamy, first observed by TREUB (34) in *Casuarina suberosa*, is recorded by BENSON (2) for *Betula, Alnus, Corylus,* and *Carpinus;* by NAWASCHIN (24, 25) for *Juglans regia* and *Corylus avellana;* by BILLINGS (4) in material of *Carya olivaeformis,* collected a few days after the withering of the anthers; and by WOODROOF and WOODROOF (37), also in the pecan. The writer's observations show that at the time of pollination the ovule is not completely surrounded by the integument, although it is later so inclosed. The pollen tube grows inward through the tissue of the stigma until near the central region of the style, and then grows downward, not in the stylar canal, but along the general course of its vascular tissues. It appears to go down to the chalazal region in various ways, usually in the wall of the ovary. In one case a pollen tube was unmistakably growing downward through the integument.

The ovule at the time of pollination is small and relatively undeveloped, and is not yet surrounded by the integument (fig. 4). Two weeks later the ovule is surrounded by the integument, the megaspore mother cell is discerned, and pollen tubes are present in the nucellus. There is considerable difference at this stage between the pollinated and unpollinated ovules. Although the evidence shows that considerable time elapses between pollination and fertilization, pollination is promptly followed by germination of the pollen grains, growth of the pollen tubes, and this in turn by progressive changes in the several ovarian and ovular tissues. Fig. 5 shows an ovule 3 weeks after pollination.

STERILITY

Sterility in plants has been the object of a considerable number of investigations, many of which indicate lack of pollination to be a factor of considerable importance. SAVASTANO (29) and STANCANELLI (31) record cases of sterility in pistache and filbert, both of

which are wind-pollinated nut fruits, where lack of pollination and fertilization was given as the cause.

There is evidence to show that sterility in the pecan is due to lack of viable pollen rather than to self- or inter-incompatibility. Regarding sterility in the pecan in Georgia, STUCKEY (32) states that self-sterility is due primarily to the interval in time between the receptive stage of the pistillate flowers and the shedding of pollen. This type of self-sterility, lack of self-pollination instead of true self-sterility or incompatibility, is not the usual condition in commercial fruit plants.

MORRIS (21) showed that eight varieties of the pecan gave an average of 74 per cent set when self-pollinated, and that most of the varieties gave a good set if the pollen was shed shortly before or during receptivity; but when shed early in the season it was not effective on the latest-maturing stigmas.

FACTORS AFFECTING FRUIT SHEDDING.—It is apparently possible to distinguish three distinct periods of dropping in the orchards at College Station: (1) at time of pollination; (2) 2–4 weeks after pollination; (3) at irregular intervals during the remainder of the growing period. With regard to the first condition, certain flower clusters shrivel and dry with the young nuts or flowers still attached to the peduncle. In other clusters, or in fact in nearly all clusters, there are one to three immature nuts at the apical end which shed along with the tip of the peduncle about the time of pollination. This last mentioned type of shedding, however, still leaves a sufficiently large cluster of nuts to produce a good crop. The shriveling of entire clusters has not been observed to any great extent, but the disturbed nutritive conditions following over-production might be responsible for such a condition, as explained by WOODROOF, WOODROOF, and BAILEY (38). The varieties Delmas and Burkett, used in this work, have borne good crops every year during the course of this study, and there has been no opportunity to observe "off" years.

The second type of dropping, referred to as the "May drop" (38), since it has been observed to occur 2–4 weeks following pollination in April and May, has proved of greatest significance in this study. WOODROOF and WOODROOF (37) state that fertilization occurs 2–4 weeks following pollination. The amount of this second

drop ranged from 11 to 52 per cent generally to 20 to 30 per cent, as indicated in table I.

The third drop later in the season, due presumably to environmental factors, does not amount to a large percentage of the total drop. The data in table I, showing the total drop to July 15 (about

TABLE I

AMOUNT OF DROP UNDER CONDITIONS OF OPEN POLLINATION 1927–1929

VARIETY	1927		1928		1929	
	Total nuts	Percentage dropped	Total nuts	Percentage dropped	Total nuts	Percentage dropped
	Four weeks after pollination					
Schley..................	119	11	....	....	....	....
Texas Prolific...........	301	10	303	22	....	....
Delmas.................	363	23	527	27	2100	17
Burkett................	383	19	498	26	1980	41
Rome..................	209	52	....	....	....	....
Success.................	87	17	....	....	....	....
San Saba...............	....	....	388	23	....	....
Stuart.................	....	....	328	23	....	....
Moneymaker............	....	....	420	20	....	....
All varieties...........	....	22	....	23	....	29
	To July 15					
Delmas.................	....	....	527	35	2100	24
Burkett................	....	....	498	36	1980	48
San Saba...............	....	....	388	26	....	....
Stuart.................	....	....	328	28	....	....
Texas Prolific...........	....	....	303	26	....	....
Moneymaker............	....	....	420	23	....	....
All varieties...........	....	....	....	29	....	36

10 weeks after pollination), and the work of BILSING (5) which shows the drop for the entire summer, indicate that the early or "May" fall may account for the greater part of the seasonal drop.

In the case of the unpollinated nuts, which were sacked before any pollen was shed and kept so until no more pollen was available, there appeared to be about the same amount and character of drop during the first 3 weeks following pollination. After this time, however, the rate of dropping increased rapidly and the condition at the

end of the fourth week is shown in table II. There were no unpollinated Burkett nuts to be found for collection the fifth week after pollination time, and, although the Delmas nuts persisted somewhat longer, there were only twelve of these unpollinated nuts after the sixth week. The latter nuts persisting at this time showed clearly defined abscission layers in all cases examined.

ABSCISSION.—Two general types of shedding were observed in Delmas and Burkett. In one case, which is less frequently observed,

TABLE II

EFFECT OF POLLINATION AND LACK OF POLLINATION ON EARLY
DROP OF PECANS, 1928

VARIETY	TOTAL NO. OF CLUSTERS	PERCENTAGE OF CLUSTERS IN WHICH NUMBER OF NUTS REMAINING AFTER 4 WEEKS WAS						
		0	1	2	3	4	5	6
		Sacked and not pollinated						
Delmas.........	392	32	7	15	20	18	7	1
Burkett.........	373	69	5	6	9	7	3	1
		Pollinated						
Delmas.........	253	0	7	21	31	28	10	3
Burkett.........	250	6	5	11	28	34	13	3

the entire peduncle dries up, although the nuts persist. This occurs in the early stages of development of the cluster, usually within a few days after pollination. The nuts never attain the size they do in the case mentioned later. Shedding of the entire peduncle is due in nearly all cases to mechanical injury, such as insect damage or breakage by wind.

The more common occurrence is the shedding of the individual flowers from the peduncle. The nuts in the cluster do not shed all at the same time, although there does not appear to be any regular order for the drop. Even after all the nuts have dropped from the peduncle, it persists in a green condition for a period of several weeks. The evidence presented by the basal region of unpollinated nuts indicates that the abscission layer is well defined within 3 weeks after receptivity. The abscission appears to be produced in the char-

acteristic manner, as described by HANNIG (9), LLOYD (12, 13), and NAMIKAWA (22, 23) for various nut fruits, not including the pecan. The walls of the cells in the separation layer are softened and the cells grow longitudinally, producing considerable displacement and disruption of the tissues.

The data presented in tables I and II indicate that the latter type of drop, the shedding of the separate nuts from the peduncle, follows pollination. The few unpollinated nuts in the controlled experiments which persisted after the 6-week period may actually have been pollinated accidentally, or may have developed parthenocarpically. There are sometimes a few seedless pecans, which might be accounted for on this basis.

EFFECT UPON SHEDDING OF PEDUNCLE POSITION.—Preliminary studies were made to determine the relative ability of the nuts in different positions on the peduncle to set and mature. A large number of clusters were treated in the following ways: (1) pollination of two basal flowers only; (2) pollination of two terminal flowers only; (3) pollination of two terminal flowers and snipping off basal flowers; (4) pollination of one basal and one terminal flower. Considerable difficulty was encountered in this work, owing to the lightness of the pollen, which made it almost impossible to keep it from the other flowers in the cluster. A number of pollinations were made as outlined, after the nuts to be left unpollinated had been covered with small gelatin capsules. Because of high winds, most of these capsules were shaken off and the nuts pollinated from loose pollen in the bags. It was also attempted to kill the stigmatic surface of the nuts not to be pollinated with osmic acid. The results in both cases were of questionable value and are not included.

Later studies on open pollinated clusters, however, show that there is a rather definite relationship between the position of the nut in the cluster and the probability of its shedding. Table III indicates that in clusters of varying numbers the basal nut or flower is much more likely to be dropped than any other. The position next to the basal is the one showing the next largest percentage of drop, and the apical nut or flower third. The percentages of drop in the respective positions are 13, 7, and 4.5. Those nuts in intermediate positions are much less likely to drop. These data are not in accord with

those given by WOODROOF, WOODROOF, and BAILEY (38), who found the number of nuts to the cluster influenced the position of shedding. It is frequently observed with the pecans at College Station, how-

TABLE III

RELATION BETWEEN POSITION OF NUT IN CLUSTER AND SHEDDING

VARIETY	No. OF NUTS	PERCENTAGE SHED TO JULY 2, 1928					
		1 (basal)	2	3	4	5	6
Clusters of 6 nuts							
Delmas	264	12	8	3	2	3	6
Burkett	156	14	9	2	4	6	4
San Saba	12	18	8	0	0	0	0
Moneymaker	48	14	4	0	2	0	2
Total	480	13	8	2	3	4	5
Clusters of 5 nuts							
Delmas	215	13	9	4	1	5	
Burkett	250	14	10	5	4	5	
San Saba	90	12	2	3	1	2	
Stuart	50	16	6	6	2	10	
Moneymaker	110	10	3	0	0	4	
Total	715	13	7	4	2	5	
Clusters of 4 nuts							
Delmas	40	17	5	2	7		
Burkett	76	20	8	4	4		
San Saba	204	11	8	1	5		
Stuart	144	8	5	5	5		
Texas Prolific	116	20	4	1	3		
Moneymaker	200	13	7	3	3		
Total	780	13	6	3	4		
Clusters of 3 nuts							
Delmas	9	11	0	11			
Burkett	15	20	7	0			
San Saba	84	12	13	5			
Stuart	87	12	6	6			
Texas Prolific	123	16	5	4			
Moneymaker	54	7	11	2			
Total	372	13	8	4			

ever, that the basal nut does not always complete its development in time for pollination, and the heavy shedding from this position may be attributed to lack of pollination.

Within the pistillate inflorescence, the different flowers of the cluster are at the same stage of development at the time of pollination, except for the smaller ones noted later. It has not been possible to fix a different time of receptivity for the different flowers of normal size in the cluster. According to WOODROOF and WOODROOF (37), the basal flowers are differentiated first, and there are always some undeveloped flowers at the apex of each cluster. These flowers are normal but not far enough advanced to be receptive, and are shed soon after the period of pollination (fig. 6). It has been observed in several cases, especially with Texas Prolific, that there are sometimes one or two immature flowers at the base of the cluster; these have always been observed to fall without reaching the stage for pollination. WOODROOF and WOODROOF (37) illustrate this characteristic, but state that such flowers may develop into nuts.

A careful consideration of the preceding data seems to warrant the following interpretations: (1) The so-called "May" drop of pecans occurring 2–4 weeks after pollination accounts for the greater portion of the total drop, except those which are damaged by insects and diseases, or drop later as the result of drought. (2) This "May" drop appears to be the result of lack of fertilization of the flowers, due primarily to lack of pollination, since it coincides with the drop of sacked unpollinated flowers. (3) A proper supply of pollen at the time the pistillate flowers are receptive is the primary requisite for setting and development of the nuts.

In view of the fact that pollination seems to be the limiting factor in determining the set of fruit in the pecan, a consideration of the factors influencing pollination is of primary importance. Since dichogamy nearly always occurs in monoecious or dioecious plants, investigations of this condition were made in the case of the pecan.

DICHOGAMY

The factors of primary importance in the consideration of dichogamy are the character and extent of the difference in time of maturity of pistillate and staminate flowers, and the factors which

influence this difference in maturity. KERNER and OLIVER (11) state that all monoecious plants are protogynous, although other workers (15–20, 27) have shown that protogyny is not always the rule in the nut fruits. STUCKEY (32) divided the pecan varieties into two groups on the basis of dichogamy, stating that in group I the pistillate flowers of most varieties become receptive at the same time that the

TABLE IV

RANGE OF MATURITY OF PECAN FLOWERS, 1925–29 (DAYS AFTER MARCH 31; E.G., APRIL 10 = 10, MAY 10 = 40)

VARIETY	STAMINATE					PISTILLATE				
	1925	1926	1927	1928	1929	1925	1926	1927	1928	1929
Old orchard										
Texas Prolific........	9–16	21–26	10–15	20–30	9–14	11–22	31–40	17–21	24–34	9–15
San Saba............	9–15	27–32	10–16	24–34	11–20	11–23	33–38	17–24	28–39	11–21
Delmas..............	14–21	31–38	17–23	31–40	20–25	8–15	32–37	19–20	23–32	10–18
Stuart...............	14–24	30–38	18–30	35–43	21–26	12–24	34–40	19–22	28–36	10–20
Moneymaker.........	17–21	31–37	14–20	29–37	16–21	10–17	31–35	14–15	24–30	11–19
Bolton..............	9–17	28–34	13–20	27–35	16–22	7–12	26–28	13–14	23–28	9–15
Rome...............	9–13	24–28	10–15	24–32	11–19	8–13	30–35	18–20	25–35	9–15
Young orchard										
Schley..............	....	....	17–28	33–39	19–24	....	....	19–21	24–34	10–18
Moneymaker.........	....	....	14–21	28–36	20–27	....	....	12–13	27–42	10–18
Success.............	....	....	26–28	32–39	17–22	....	....	26–30	25–34	13–19
Alley...............	....	....	11–21	24–32	11–16	....	....	22–25	31–45	14–20
Moore..............	....	....	8–14	22–30	7–13	....	....	21–26	25–44	10–14
Burkett.............	....	....	17–28	32–40	20–26	....	....	18–21	24–32	9–17
Onliwon............	....	....	11–19	26–33	14–18	....	....	24–30	28–44	11–16
Atwater............	....	....	....	33–40	20–25	....	....	....	25–32	10–14
Western Schley......	....	....	15–20	29–34	14–19	....	....	24–28	32–46	11–16
Delmas.............	....	....	17–30	32–41	20–26	....	....	19–21	25–37	10–20

staminate flowers shed their pollen, while in group II the pistillate flowers become receptive 2–10 days before the staminate flowers shed their pollen.

The blossoming data in table IV show that the type of dichogamy in the pecan is not always fixed, and data in table V make it evident that there is a strong tendency in certain seasons toward protandry and in others toward protogyny. Some of the most important commercial varieties, as Delmas, Schley, Stuart, and Burkett, respond

to these seasonal tendencies. It is also shown (table V) that there is a group of varieties which have a positive tendency toward protandry, which tendency has not been observed by previous investigators. Texas Prolific, San Saba, Moore, and Alley were protandrous every year. Another group shows a positive trend toward protogyny, although this is not so strongly marked as in the protandrous

TABLE V

CHARACTER AND EXTENT IN DAYS OF DICHOGAMY; + INDICATING PROTOGYNY AND − PROTANDRY

VARIETY	1925	1926	1927	1928	1929
	Old orchard				
Texas Prolific............	2−	10−	7−	4−	0
San Saba................	2−	6−	9−	4−	0
Delmas.................	6+	1−	2−	6+	10+
Stuart..................	2+	4−	1−	6+	11+
Moneymaker............	7+	0	0	5+	5+
Bolton.................	2+	2+	1+	4+	7+
Rome..................	1+	6−	8−	2+	4−
	Young orchard				
Schley..................	....	....	2−	9+	9+
Moneymaker............	....	....	2+	1+	10+
Success.................	....	....	0	7+	4+
Alley...................	....	....	11−	7−	3−
Moore..................	....	....	13−	3−	3−
Burkett.................	....	....	1−	8+	11+
Onliwon................	....	....	13−	2−	3+
Atwater................	....	....	....	8+	10+
Western Schley..........	....	....	7−	3−	3+
Delmas.................	....	....	2−	7+	10+

group. Moneymaker, Success, and Bolton were protogynous in almost every case through the entire period; Moneymaker in 1926 and 1927, and Success in 1927, matured staminate and pistillate flowers on the same date.

In view of these facts, it seems advisable to depart from previous classifications, and make three groups of pecan varieties as regards dichogamy: protandrous, fluctuating, and protogynous.

SEASONAL TENDENCIES.—The behavior of the fluctuating varieties, as well as the tendency of the positive varieties toward over-

lapping in blossoming, indicate that the seasons of 1925, 1928, and 1929 exerted some influence toward protogyny; the seasons of 1926 and 1927 toward protandry. It was considered advisable to ascertain whether these tendencies might be associated with definite conditions of environment, as suggested by MEEHAN (16, 18).

The critical factors which might cause a difference in maturity of the flowers were considered to be temperature and rainfall, although

TABLE VI

WEEKLY, MONTHLY, AND SEASONAL ACCUMULATION OF HEAT UNITS ABOVE 40° F. (MEAN) AT COLLEGE STATION, 1925–29

DATE		1925	1926	1927	1928*	1929
January	7.............	72.5	51.0	113.0	20.0	40.5
	14.............	123.0	74.0	166.0	136.5	110.0
	21.............	159.0	188.5	269.0	279.5	229.0
	28.............	231.0	207.0	326.0	228.0	321.0
February	4.............	320.5	309.5	490.5	460.0	368.0
	11.............	483.5	432.5	602.5	570.0	387.5
	18.............	597.5	592.5	729.0	625.5	445.5
	25.............	781.5	728.5	857.5	686.5	494.0
March	4.............	901.5	853.0	923.0	781.5	599.5
	11.............	1103.0	982.0	1069.0	961.5	750.0
	18.............	1272.5	1067.0	1224.0	1119.0	873.0
	25.............	1441.0	1249.0	1322.5	1241.5	1076.5
April	1.............	1644.0	1316.5	1524.0	1422.0	1314.0
	8.............	1843.5	1487.5	1742.0	1590.0	1573.0
	15.............	2081.0	1621.5	1977.5	1684.0	1786.0
	22.............	2358.0	1799.5	2173.0	1857.5	2016.0
	29.............	2618.5	1982.0	2340.0	2020.5	2261.5
	30.............	2632.5	2010.0	2372.0	2079.5	2302.0

* Leap year, February 29 included in figures and date advanced one day, beginning March 3.

wind and atmospheric humidity probably have considerable influence upon duration of receptivity of the stigmas and shedding of the pollen.

In the consideration of the effect of temperature, the number of heat units above 40° F. mean was recorded for the first 4 months of each year, as described in the first part of this work. These figures were arranged to show weekly, monthly, and seasonal accumulations during the entire period (table VI). As shown by the totals on April 30, the year 1925 had the greatest number of heat units; 1927 and

1929 were about equal and considerably fewer than 1925; and 1926 and 1928 had still fewer than the two last mentioned.

The monthly rainfall for the entire period and the weekly accumulations are shown in table VII. The rainfall in the three protogynous seasons was considerably less than in the protandrous seasons. For the protogynous seasons, 1925 had 4 inches, 1928 had 9 inches,

TABLE VII

WEEKLY, MONTHLY, AND SEASONAL RAINFALL AT COLLEGE
STATION (INCHES) 1925–29

DATE	1925	1926	1927	1928	1929
January					
7..................	0.00	1.55	0.00	0.29	2.53
14..................	1.12	1.58	1.03	0.29	4.50
21..................	1.69	3.97	1.30	0.35	4.84
28..................	1.69	4.32	1.63	0.39	5.09
February					
4..................	1.72	4.37	1.63	1.47	5.24
11..................	1.77	4.37	3.44	1.61	5.90
18..................	1.77	4.58	3.58	2.35	5.95
25..................	2.17	4.58	3.58	4.70	6.26
March					
4..................	2.34	5.12	7.88	4.70	1.92
11..................	2.34	7.80	9.07	5.97	7.04
18..................	2.67	8.48	9.07	6.61	7.99
25..................	2.67	10.46	10.76	6.69	8.92
April					
1..................	3.55	12.62	10.76	6.99	8.94
8..................	3.55	12.69	10.77	7.64	9.71
15..................	3.55	13.96	14.69	9.74	10.68
22..................	3.55	16.37	16.91	9.83	10.69
29..................	4.17	16.61	17.39	9.83	10.69

and 1929 had 10 inches. For the protandrous years, 1926 had 17 inches, and 1927 had 17 inches. The weekly accumulations give a better idea as to the distribution of this rainfall.

When the combined effect of temperature and rainfall is considered, it may be observed that these two factors compensate for each other to some extent. The season of 1925, which was very hot and very dry, was not so strongly protogynous as the season of 1928, which was much cooler and had more rainfall. In the same way the season of 1929, which was even more strongly protogynous than 1928, had very little more rainfall than 1928, and was much cooler up to the early part of April. The greater total of heat units is ac-

counted for by the sustained high temperature in April, after many of the trees had blossomed.

In the two protandrous seasons of 1926 and 1927, however, there was no observed difference in blossoming which might be attributed to the difference in heat unis. The heavy rainfall is the only outstanding factor which is common to these two seasons.

When the heat units to date of maturity are considered, as shown in table VIII, two facts stand out:

1. On the basis of the means, there is a much greater variation in any one year between varieties in number of heat units to maturity of staminate flowers than to maturity of pistillate flowers. To express the same behavior in a different way, there is less difference in the date of blossoming of pistillate flowers than of staminate. This same difference is apparent in both the old and the young orchards, where both age and variety of the trees are different. The significance of these differences is greater from the fact that Delmas and Moneymaker, the only two varieties occurring in both orchards, check closely in their requirements.

2. The pistillate flowers vary much more from year to year in their total requirements than do the staminate flowers, the greatest coefficient of variability for staminate flowers, 6.1, being less than the least coefficient for pistillate flowers, 6.7. The actual range in the coefficient of variability for the staminate flowers of the different varieties is from 2.3 to 6.1, and for the pistillate flowers, 6.7 to 9.5. As shown in table IX, the odds in favor of the significance of this difference are 999 to 1.

With regard to all the observations recorded, it may be stated that the conditions of environment in the spring exert considerable influence on blossoming of the pecan. In general it appears that, although the date of maturity of both staminate and pistillate flowers is influenced by favorable conditions for growth in the spring, the staminate flowers respond more readily than the pistillate flowers. This fact would indicate a possibility that seasons favorable for early growth might be favorable for protandry. ADRIANCE (1) has previously presented data showing some effects of spring temperatures in this respect.

In confirmation of this idea, it may be stated that since the pistillate flowers of the pecan are differentiated in the same spring that

TABLE VIII

HEAT UNITS TO DATE OF MATURITY OF FLOWERS

Variety	Staminate								Pistillate							
	1925	1926	1927	1928	1929	Mean	S.D.	C.V.	1925	1926	1927	1928	1929	Mean	S.D.	C.V.
Old orchard																
Delmas	2010	2010	2004	2079	1949	2010	±14 46.1	2.3	1811	2040	2068	1926	1638	1896	±53 176.7	9.2
Moneymaker	2117	2010	1924	2020	1816	1977	±34 113.2	5.7	1875	2010	1924	1905	1667	1876	±38 127.3	6.7
Texas Prolific	1843	1746	1786	1789	1604	1753	±27 90.4	5.1	1908	2010	2004	1905	1604	1885	±50 165.5	8.8
San Saba	1843	1904	1786	1905	1667	1821	±29 99.2	5.4	1908	2070	2068	2002	1667	1943	±50 167.8	8.6
Stuart	2010	1982	2035	2199	1986	2042	±27 89.3	4.3	1939	2100	2068	2020	1638	1953	±56 186.2	9.5
Bolton	1843	1930	1889	1980	1816	1891	±20 65.9	3.4	1782	1881	1856	1885	1604	1801	±35 117.9	6.5
Rome	1843	1820	1786	1980	1667	1821	±33 112.7	6.1	1811	1982	2035	1926	1604	1871	±51 171.1	9.1
Young orchard																
Delmas	....	....	2004	2119	1949	2024					2068	1926	1638	1877		
Moneymaker	....	....	1924	2002	1949	1958					1856	1980	1638	1825		
Schley	....	....	2004	2139	1909	2017					2068	1905	1638	1870		
Success	....	....	2219	2219	1845	2061					2219	1926	1725	1957		
Alley	....	....	1822	1905	1667	1798					2162	2079	1756	1999		
Moore	....	....	1708	1857	1540	1702					2138	1926	1638	1901		
Burkett	....	....	2004	2119	1949	2024					2035	1905	1638	1859		
Onliwon	....	....	1822	1951	1756	1843					2185	2002	1667	1951		
Atwater	....	....	....	2139	1949	2044						1926	1638	1782		
Western Schley	....	....	1952	2020	1756	1909					2185	2109	1667	1987		

they appear, but staminate flowers are differentiated the previous spring, under these conditions apparently a "quick" season might mature the staminate flowers earlier than the pistillate. A cold or dry season, on the other hand, might retard the opening of the staminate flowers long enough for the pistillate to differentiate.

TABLE IX

SIGNIFICANCE OF COEFFICIENTS OF VARIABILITY IN TABLE VIII

C.V. STAMINATE A	C.V. PISTILLATE B	B − A	D	D²
5.1............	8.8	3.7	0.0	0.0
5.4............	8.6	3.2	−0.5	0.25
2.3............	9.2	6.9	3.2	10.24
4.3............	9.5	5.2	1.5	2.25
5.7............	6.7	1.0	−2.7	7.29
3.4............	6.5	3.1	−0.6	0.36
6.1............	9.1	3.0	−0.7	0.49
		7)26.1	+4.7	7)20.88
		3.7	−4.5	2.98

$$P = \sqrt{2.98}$$
$$= 1.726$$
$$Z = \frac{3.7}{1.726}$$
$$= 2.1436$$

odds 999 to 1.

A further consideration is the fact that pistillate flowers are borne on the terminal part of new shoots, and a season favorable to strong vegetation might delay their development.

Summary

1. The pistillate flower of the pecan consists of an orthotropous ovule, surrounded by a single integument. The portion which becomes the shell consists of two carpels, which are transverse on the axis of the inflorescence. The 4-valved husk is developed from the lower portions of the calyx lobes. The flowers are sessile on the peduncle, and are borne in clusters usually of two to six.

2. The pollen tube grows down through the style and ovary wall or integument to the base of the ovule, and returns through the chalaza and nucellus to the embryo sac, fertilization occurring about 4 weeks after pollination.

3. There is a definite drop of young nuts about 4 weeks after the

time of pollination, and this drop accounts for over 75 per cent of the seasonal drop. It appears to be due to lack of pollination.

4. The varieties tested show no evidence of self-incompatibility or inter-incompatibility. Good sets of fruit were obtained from any variety of pollen available when the flowers were receptive.

5. The period of maturity of staminate and pistillate flowers of the pecan do not often coincide. This condition of dichogamy may be complete or incomplete, and the special type may be protandry or protogyny. Some varieties, such as Moore, Alley, Texas Prolific, and San Saba, have been protandrous every season, and have had pollen available in time to pollinate the earliest flowers of any variety. Some varieties, especially Moneymaker, Bolton, and Success, have been protogynous or overlapping slightly every year, and have been dependent upon other varieties for pollination in almost every case. A group of varieties, including Delmas, Burkett, Schley, and Stuart, which are frequently recommended for planting together, have been protandrous or overlapping only two years in five. These leading varieties in Texas need other varieties near them to insure availability of early pollen.

6. Certain seasons have been favorable to protandry and others to protogyny. The data on temperature and rainfall for the spring seasons indicate that moisture and high temperature in this period favor early maturity of the staminate flowers, and cool, dry seasons favor earlier maturity of the pistillate flowers. The warm seasons of heavier rainfall advance maturity of both the staminate and pistillate flowers, but the staminate flowers of any one variety are less variable in their requirement of heat units.

7. It may be said that the early dropping of fruits in the pecan is due primarily to lack of pollination, and this, in turn, is due to dichogamy. Certain varieties (Moore, Alley, Texas Prolific, and San Saba) have proved to be reliable in the production of pollen at an early date, and some of these varieties should be used in every pecan orchard.

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RESPIRATION OF THE SHOOT AS AFFECTED BY TEMPERATURE CHANGES OF THE ROOT[1]

W. H. MICHAELS

(WITH FIVE FIGURES)

Introduction

The effect of temperature upon plant respiration has been extensively studied since the work of DE SAUSSURE and GAREAU, both of whom simply demonstrated that the rate of respiration rose with an increase in temperature. CZAPEK (8), KUIJPER (13), and FERNANDES (10) give extensive reviews of the literature dealing with this phase of respiration. In general it may be said that the rule of Van't Hoff holds for plant respiration between certain limits of temperature, usually considered to be about $5°-25°$ C. There are a number of exceptions to this rule, however, for example, BENNETT and BARTHOLOMEW (2) found the respiration of potato tubers to be greater at $0°$ than at $5°$ C., also that the rule appeared to hold in this case for the rather wide limits of $5°-45°$ C.

The rate of respiration is by no means constant, even at temperatures which may be optimum for the growth of the organ under investigation. MATTHAEI (18), KUIJPER (13), and FERNANDES (10) have reported marked fluctuations in the respiratory rate, especially at temperatures above $30°$ C. FERNANDES pointed out that the temperature at which these fluctuations become apparent varies with the age of the seedling, and that the response to temperature varies with different lots of seedlings, even though they be of the same age. BLACKMAN and PARIJA (4), working with individual apples at $22°$ C., were forced to use two "contour lines" in the graphs presenting their data, in order to indicate the magnitude of the fluctuations in the respiratory rate.

PALLADIN (19), experimenting with etiolated shoots of *Vicia faba*, showed that sudden temperature changes as such acted as stimuli

[1] Abstract of thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany in the Graduate School of the University of Illinois, 1929.

upon the respiration rate. WOLKOFF and MAYER (24) had earlier noted that a sudden fall in temperature caused a distinct decrease in the respiratory rate below that normally found at the lower temperature, but a sudden rise had no effect. Gradual fluctuations about a mean had no apparent influence. DETMER (9), ZIEGENBEIN (25), KUIJPER (13), and BLANC (5) all failed to find a stimulation in the respiratory rate as the result of sudden temperature change. They believe the rate of respiration to change gradually with the temperature.

That the temperature of the roots affects the growth of the shoot has been conclusively shown by BIALOBLOCKI (3), BURKHOLDER (6), CANNON (7), and many others, particularly the observations of LINFORD (16), TISDALE (23), and other plant pathologists using the Wisconsin soil-temperature tanks in their studies of the influence of the environment upon the plant and its susceptibility to disease. In general, plants growing in a soil at moderately low temperatures have a larger root system and a coarser heavier shoot than do plants grown at the same air temperature but with a higher soil temperature. The effect of a temperature shock upon the roots in relation to the behavior and future development of the plant does not appear to have been investigated.

LEHENBAUER (14) has briefly but adequately reviewed the work on the effect of sudden temperature changes upon plant growth. It seems established that a sudden temperature change deranges the metabolism of the plant, resulting in either a depression or an acceleration of the growth rate, depending upon the direction and magnitude of the temperature change and also to some extent upon the period of exposure.

Material and methods

Seeds of *Phaseolus vulgaris* (Burpee's Stringless Greenpod) were carefully selected for soundness, and measured to secure uniformity of size. These beans were then germinated in clean quartz sand at 45 per cent of the water-holding capacity. At the end of the third day at 22° C., and before the secondary roots appeared, the seedlings were taken from the sand and their seed coats removed. The roots were carefully thrust through the holes in some no. 5 one-holed rub-

ber stoppers, which were supported over tap water by means of wooden frames. The container in which the seedlings were growing was covered with a larger one that provided ample ventilation and yet shut out the light. All of the seedlings were grown in tap water, which was frequently changed. When the plants were 6–7 days old and their hypocotyls had elongated sufficiently to extend through the stoppers, cotton was packed around them to hold them in posi-
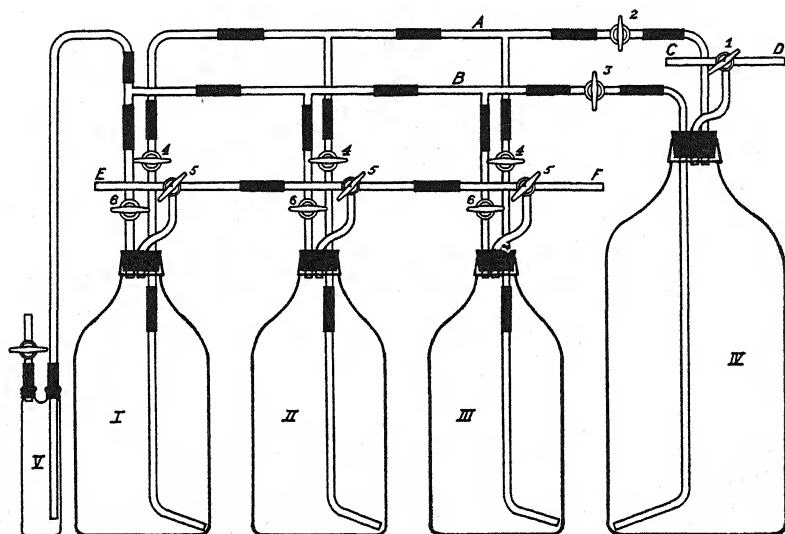

FIG. 1.—Gas collection apparatus

tion. The seedlings were left in this manner until they were needed for experimentation.

The aspirator and gas collection apparatus consisted of two sets of four bottles each, arranged as shown in fig. 1. A vacuum tank, exhausted by an automatically regulated electrically driven pump, maintained the uniform vacuum necessary for suction.

The gas collection apparatus was filled with a liquid which absorbed little or no carbon dioxide. The solution used was that of Layng and Crum, as reported by HOTTES and HAFENRICHTER (12). This liquid consists of a 35 per cent aqueous solution of zinc sulphate to which 14 gm. of concentrated sulphuric acid per liter of solution is added. Considerably more pressure or suction is needed to force

or draw this liquid through the apparatus than is required for water, but in spite of this difficulty it has proved very satisfactory.

Each of the bottles I, II, and III has a capacity of 4 liters, while the capacity of bottle IV is 12 liters. Stopcocks 2, 3, 4, and 6 all have a 2 mm. bore. It was found necessary to use stopcocks of this size when it was desired to aspirate at the rate of 3 or 4 liters in 10 minutes; at slower rates of aspiration stopcocks of 1 mm. bore should prove satisfactory. The three-way stopcocks, which serve for the passage of gas only, are of 1 mm. bore. Heavy pure gum rubber tubing was used on all joints, the rubber connections and stoppers being wired into place. Bottle V served as a safety valve.

To aspirate, stopcock 1 is turned to connect bottle IV with the vacuum through D, and stopcock 2 opened. Next stopcock 4 is opened on the bottle in which it is desired to collect the gas, in this case bottle I. The three-way cock 5 is then turned to connect the bottle with the respiratory chamber through E. The vacuum of 6 cm. now draws the liquid out of bottle I through tube A into bottle IV, and as the level of the liquid falls in bottle I the gas from the respiratory chamber is drawn into it. The speed of aspiration may be governed by the insertion of a piece of capillary tubing of the proper bore into tube A, between stopcock 2 and bottle IV. At the end of the period of gas collection stopcock 4 is closed and 5 turned to seal bottle I but to permit a through passage from E to F. For continuous gas collection, stopcocks 4 and 5 on bottle II should be opened before cocks 4 and 5 on bottle I are closed. With tight connections no solution can be withdrawn from bottle II until cock 5 on bottle I is turned to permit a through passage from E. If it is desired to have a period of aspiration between gas collections, it can easily be accomplished by attaching the vacuum, properly reduced, to F. In any case it is best to have the vacuum attached here at all times, to minimize the chances of inadvertently admitting air to the system by turning the wrong stopcock. The same procedure is then followed with bottles II and III.

To measure the volume of gas collected and to take a sample, the tube connecting the respiratory chamber with E is removed and replaced by one leading to the gas meter. The tube connecting with the vacuum at F must either be removed or the vacuum shut off.

Stopcock 2 is now closed and cock 1 turned to connect with the air pressure line through C. The air pressure was maintained at 150 gm. per square centimeter. Stopcock 3 is opened, followed by stopcock 6 on bottle I, and lastly cock 5 is turned to connect bottle I with the gas meter through E. As the liquid is now forced out of bottle IV into bottle I, the gas is forced out of bottle I through the meter. The sample was taken as the gas left the meter.

The gas meter used was one designed especially for respiration work, and was graduated to hundredths of a liter but permitted accurate estimates to thousandths. The samples were taken with the type of sampler described by BAILEY (1), and the gas analysis was made with BAILEY's modification of the Haldane-Henderson gas apparatus. All gas volumes were reduced to the dry state at 0 °C. and 760 mm. mercury pressure. The weight of carbon dioxide in milligrams was obtained with the aid of the table of densities given by PARR and KING (20). Five place logarithms were used throughout.
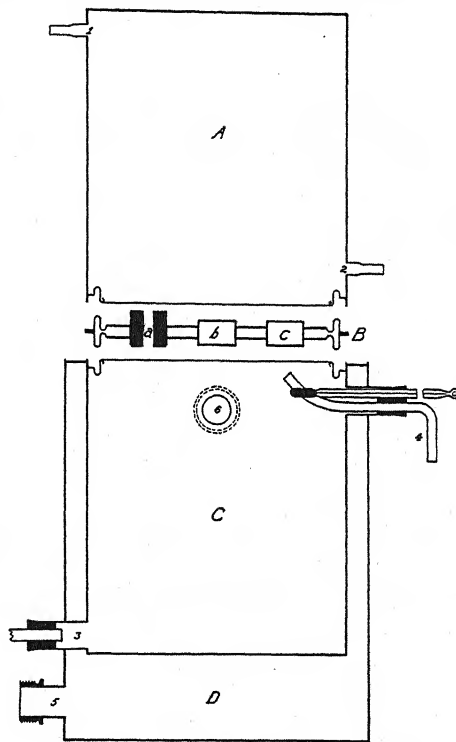

FIG. 2.—Respiration chamber: A, respiration chamber proper (1, 2, inlet and outlet respectively); B, double lid (a, b, c, brass tubes through lid and a rubber stopper inserted in a); C, inner root chamber (3, inlet and outlet tube; 4, overflow tube with thermometer inserted above); D, outer root chamber (5, 6, hose connections through which cooling liquid was circulated between C and D).

The respiration chamber is shown diagrammatically in fig. 2. It consisted of two parts, the respiration chamber proper (A), and the chamber in which the roots were held (C). Both were good quality

4-liter tin cylinders with tightly fitting pry lids. The lids were soldered together (*B*), seven holes punched through them, and short brass tubes soldered into these openings (*a*, *b*, etc.). The plants in their no. 5 stoppers were later forced into these tubes. The respiration chamber was thus inverted over the root chamber, which was soldered into a larger tin cylinder (*D*), making a double-walled chamber. Water or brine was circulated in the space between the two cylinders to control the temperature of the roots. An opening (3) was also provided in the root chamber, by means of which the water in which the roots were growing could be rapidly changed. The second opening in the root chamber carried an overflow tube (4) and a thermometer. The respiration chamber proper had two openings, an inlet tube (1) near the upper end of the cylinder and an outlet tube (2) near its lower extremity.

By means of this apparatus it was possible to increase or decrease the temperature of the water in which the roots were growing in about 6 minutes. During the entire test the respiration chamber was kept in one of the constant temperature cases which are standard equipment in the laboratory. The range of temperature fluctuation within the case was 1°. The air entering the respiration chamber was drawn through a Truog absorption tower filled with shell-caustic and then through a Winkler tube filled with 40 per cent potassium hydroxide.

When ready for an experiment, the seven selected seedlings in their rubber stoppers were forced into the holes in the double lid and the cotton removed from around the plants. Next the plants were sealed into the stoppers with chewing gum. Gum was the only seal found which did not injure the plants but would withstand the necessary vacuum. After the gum had set, the rim of the lid was lightly greased with stopcock lubricant and the respiration chamber proper forced into place. The lid was then loosened from the lower can and the chamber tested under 2.5 cm. vacuum for about 5 minutes. The respiration chamber was then placed in the temperature case and the connections with the aspirator and absorption tubes made.

The plants were always placed in the case at five o'clock of the afternoon before the test and a slow rate of aspiration maintained

all night. The actual test was begun either at seven or eight o'clock the following morning. At the end of the experiment the plants were removed from the chamber, any adhering gum washed off with alcohol, and the plants placed in the drier in preparation for the dry weight determination. All of the experiments were run in duplicate or triplicate, and the data are recorded on the basis of the dry weight of the seedlings at the end of the experiment. A total of 25 experiments, using seven seedlings each, were run. The values recorded in table I represent the averages of 194 carbon dioxide determinations and approximately 500 gas analyses.

Experimentation

The data for each temperature series are presented in table I and figs. 3, 4, and 5. The dotted horizontal line through each graph represents the total average rate of respiration per hour per gram dry weight
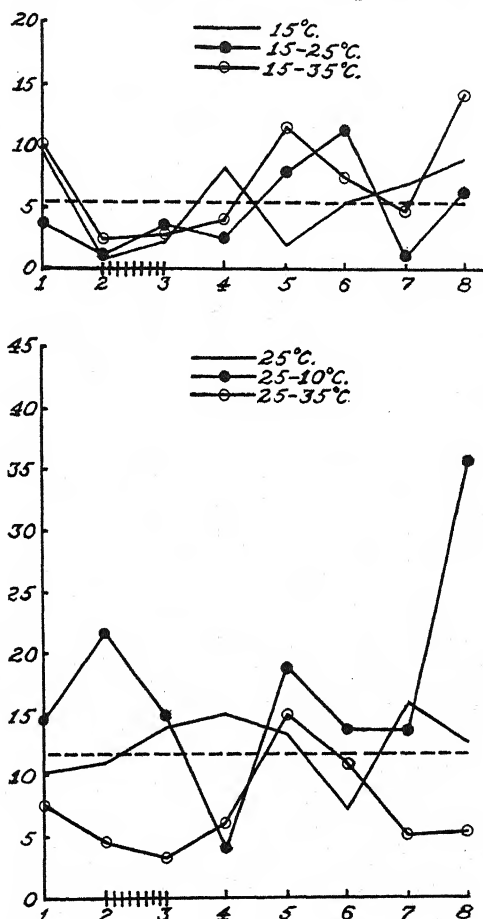
FIGS. 3, 4.—Fig. 3 (above), respiration rates of seedlings of 15° C. group; fig. 4 (below), respiration rates of 25° C. group of seedlings; all values from table I (abscissae represent time in hours; cross-hatched portion indicates time roots were at second temperature; ordinates represent mg. CO_2 emitted per hour per gm. dry weight).

at the constant temperature of the group. These average rates of respiration are 5.34 mg. of carbon dioxide per hour at 15° C.,

11.43 mg. of carbon dioxide at 25° C., and 8.35 mg. of carbon dioxide per hour at 35° C. In those experiments in which the temperature of the water in which the roots were growing was changed, the change was made at the beginning of the third hour and the return to former temperature was at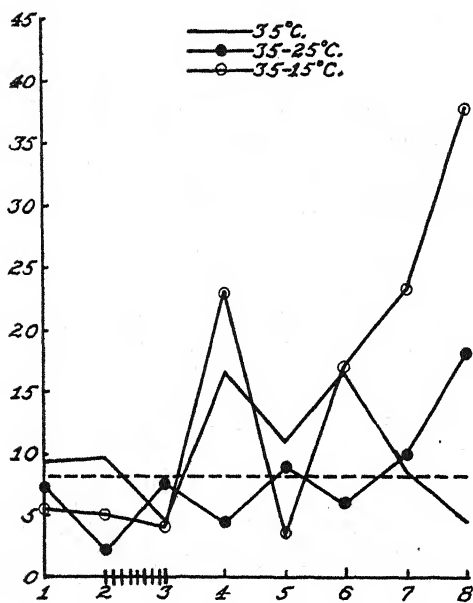 the beginning of the next hour. The cross-hatching of the abscissae indicates the period during which the roots of the plants were at the changed temperature. In the designation of any series in which two temperatures are used, the second temperature indicated always refers to the one to which the roots were changed during the third hour.

FIG. 5.—Respiration rates of seedlings of 35° C. group, all values from table I (abscissae represent time in hours; cross-hatched portion indicates time roots were at second temperature; ordinates represent mg. CO_2 emitted per hour per gm. dry weight).

15° C. GROUP.—The data for the 15° C. group are shown graphically in fig. 3. A striking feature is that all of the curves show a falling respiratory rate for the first 2 hours. The most logical explanation would appear to be that there had been a slight accumulation of carbon dioxide in the respiration chamber during the night, due to a slower aspiration rate; however, since the same rate was used throughout all of the experiments, one would expect the same type of declining curve at 25° and 35° C., but it is present in only one or two cases at these temperatures. The writer is unable to explain the coinciding of these curves. The 15° C. constant temperature curve is notable mainly for its rather wide fluctuations from the mean. The chief difference noticeable between the 15°–25° and 15°–

35° C. curves is that the former rises during the time that the roots are at the warmer temperature, while the latter remains at a uniform level. The shock of the change to 35° C. seems to have been severe enough to cause the respiratory process to remain on a level with that of the previous hour. The 15°–25° C. curve, on the other hand, rises during the third hour, probably because the temperature change is too small to depress the respiration rate for the entire hour. This curve shows a decrease in the respiration rate for the fourth hour, apparently due to the effect of the sudden change back to 15° C. The 15°–35° C. curve shows no signs of a second shock.

TABLE I

Respiration rates of etiolated bean seedlings, 7–9 days old, when subjected to different temperature treatments, in mg. carbon dioxide per hour per gm. dry weight

Hours of experimentation	15° C. GROUP			25° C. GROUP			35° C. GROUP		
	15°	15°–25°	15°–35°	25°	25°–10°	25°–35°	35°	35°–25°	35°–15°
1.......	9.48	3.58	10.26	10.19	14.29	7.59	9.28	7.84	5.37
2.......	0.77	1.05	2.47	10.93	21.67	4.64	9.68	2.25	5.04
3.......	2.02	3.52*	2.69*	13.92	14.90*	3.26*	4.58	7.89*	4.02*
4.......	8.13	2.56	4.17	15.09	4.03	6.07	16.44	4.23	23.15
5.......	1.97	7.97	11.56	13.08	18.90	14.94	11.03	8.73	3.31
6.......	5.44	11.65	7.52	7.16	13.66	10.93	16.51	6.07	16.74
7.......	7.05	0.89	4.77	15.82	13.66	5.04	8.48	10.02	23.35
8.......	9.02	6.33	14.49	12.67	35.74	5.42	4.31	18.06	37.56

* Hour at the changed temperature.

The difference between these two curves is more easily understood when it is remembered that 25° C. is optimum temperature for the bean plant.

25° C. GROUP.—Fig. 4 presents the averages for the 25° C. group. All of the curves for the experiments in which the temperature was changed show a fall during the third hour, which in the 25°–10° C. series is continued in the fourth hour. That the temperature change acted as a stimulus is indicated by the remarkable rise in the eighth hour of the 25°–10° C. series. Changing the roots from 25° to 35° C. flattened the curve between the second and third hour. The curve then rises, the maximum rise being during the fifth hour.

35° C. GROUP.—The greatest fluctuations in the respiration rate of the entire study are found in this group. Fig. 5 presents the curves

for the various series. The large stimulation shown by the 35°–15° C. curve is probably due to the 20° difference between the two temperatures used. Again the temperature change has flattened the curve for the hour at the changed temperature, and is followed immediately by a rapid rise. The 35°–25° C. curve indicates that this temperature change had little effect other than to depress the respiration rate for the four or five hours following the change. It is impossible to say that there was any shock effect in this case.

Discussion

These experiments indicate that sudden temperature changes upon the roots of etiolated bean seedlings produce a shock which is transmitted to the shoot, as evidenced by its changed respiratory rate. The magnitude of the shock does not appear to be in proportion to the difference between the two temperatures employed. This is brought out by a comparison of the curves for the 15°–35°, 25°–10°, and 35°–15° C. series. The 15°–35° and 35°–15° C. series received shocks of identical magnitude, yet the former shows comparatively little shock effect while the latter shows the greatest effect of the entire group of experiments. Further, the shock shown by the 25°–10° series is greater than that for the 15°–35° C. series. These facts seem to indicate that the direction of the shock is more important than its magnitude.

In a general way this conclusion is borne out by PALLADIN's (19) results. He found an increase of 53 per cent in the amount of carbon dioxide given off over that of the control series, when the etiolated *Vicia faba* shoots floating on saccharose solution, which had been at a temperature of 36°–37.5° for several days, were tested at 18°–22° C. Similar shoots floating on saccharose solution transferred from 7°–12° to 18°–22° C. showed an increase of only 40 per cent in their carbon dioxide output. In this case the range of temperatures was also different, however, there being about 18° difference between the medium and high temperatures, and only about 9° C. difference between the medium and low temperatures. The response was certainly not in proportion to the temperature difference, but it is impossible to say definitely that the direction of the change influenced it.

A study of the graphs of the various series shows that the $25°$ curve has fewer sharp fluctuations than either the $15°$ or the $35°$ C. curves. The $25°$ plants were at a temperature which is very near their optimum temperature for growth. Apparently their metabolic processes were going on at a uniform rate, the equilibrium of which is hard to disturb. This was not true for the other two groups. These results are contrary to the work of most investigators, who have found that the rate of respiration is more uniform at lower temperatures than it is at medium or high temperatures.

The type of response to the temperature change also varies. Thus in the $15°$ group and the $25°$–$35°$ C. series the curves showing the respiration rate are comparatively flat from the second to the fourth hours. For the $25°$–$10°$ and the $35°$–$15°$ C. series the curves begin to fluctuate after the third hour instead of the fourth. These last two series also show the greatest range of fluctuations of the entire group of experiments, consequently the difference in type of response must also vary with the effectiveness of the stimulation.

The marked depression in the average respiratory rate at $35°$ C. seems largely due to the action of a "time factor." It must be remembered that the average respiratory rates, indicated by the dotted lines in figs. 3, 4, and 5, represent the rate of respiration after approximately 14 hours' exposure to the respective temperatures. The magnitude of the depression may be seen when the values of Q_{10} for the present data are compared with the results obtained by KUIJPER (13) for pea seedlings, and by LUNDEGARDH (17) for the leaves of the potato plant. KUIJPER's respiration results are based on the first hour at these temperatures, while those of LUNDEGARDH cover a period 5–17 minutes. These results are:

Temperature	$15°$ C.	$25°$ C.	$35°$ C.
Q_{10} of present data		2.14	0.70
Q_{10} of KUIJPER's data		2.34	1.58
Q_{10} of LUNDEGARDH's data		2.20	1.30

Plainly the value of Q_{10} for the $10°$ between $25°$ and $35°$ C. in the present experiments is much lower than in the other two cited.

The present experiments also indicate clearly the close connection between the activities of the shoot and those of the root. By just what means a shock administered to the cells of the root is trans-

mitted to the cells of the shoot is impossible to say at present. Snow (21) and others have advanced the idea that a hormone or similar substance is formed as the result of a stimulus, and is then carried to all parts of the plant. Others believe that the stimulus is of an electrical nature, and passes from cell to cell by means of the slender connecting strands of protoplasm or plasmodesma.

Regardless of the method of conduction, it is certain that in this case a stimulus acting upon the root has affected the metabolism of the shoot. From this it might be concluded that a stimulus acting upon one part of the plant will influence the entire plant. That this is not always the case is illustrated by the various treatments used to break up the rest period of woody plants, in which only the portion of the plant actually treated has its rest period broken. Similarly, Garner and Allard (11) have found localized response to length of day in cosmos. It is realized that these cases are not exactly comparable with the present experiments, for here we are dealing with the effect of a stimulus upon a portion of a plant which could be separated from the remainder of the organism without seriously injuring it. It seems, therefore, that the effect of a stimulus acting upon the entire root or shoot is different in respect to transmission from the effect if it had acted upon only a small portion of the plant.

The action of the stimulus may perhaps best be understood by a consideration of the cell and its metabolism. A number of investigators consider the cell as a colloidal complex in which the various chemical reactions proceed. Spoehr (22) has expressed this concept as follows:

It seems highly improbable that the life processes consist of any one series of chemical changes or are dependent upon any particular molecule or chemical group. But rather the simple life processes entailing energy changes may perhaps be regarded as a complex of interrelated chemical changes taking place in a certain medium or substratum. This medium, colloidal dispersion, mixture, or aggregate of various substances is the seat or substratum in which the various chemical reactions take place, the nature and course of which are determined by the complex of properties associated with water control, surface phenomena, and of course catalysts (such as inorganic salts and enzymes). These colloids do not enter into or support the chemical reactions or do so probably only in a rather indirect manner, serving primarily as a physical medium. Such a system would be of a heterogeneous nature and, of course, of the most complex type,

and capable of various adsorption phenomena productive of localized action which would influence the function as well as the structure of the system. In fact, such a hypothesis would demand that the substratum be relatively stable, that the colloidal material once formed does not break down as readily as the other substances, or only after the supply of these has been exhausted. This does not mean actual or chemical stability, but rather relative to the other substances under the existing physiological conditions, as, for instance, relatively slightly dissociated by salts or other catalysts and resistant to the action of enzymes.

Plant respiration is at present conceived to be a complicated life process which takes the form of a chain of catalytic reactions in which the products formed by one reaction become the reactants in the next link. These reactions, in general, follow the law of mass action which in its simplest form states that the rate at which any reaction proceeds is directly proportional to the amount, or rather the concentration, of the reacting substance. In other words, any change in the amount of one of the reactants brings about the establishment of an entirely new equilibrium, with a resulting increase or decrease in the amount of end product. Further, there is the probability that the individual steps may respond differently to external influences, for example, they might have different temperature coefficients, in which case a rise in temperature might accelerate one link more than the others, resulting in an excess of its end product; or a lowering of the temperature might retard one reaction more than the others. This reaction would then become the limiting factor in the series.

Does the stimulus of these experiments act directly upon this chain of reactions by changing the speed of one or more links, or does it act indirectly by first influencing the structure of the protoplasm? These two viewpoints are not necessarily opposed. Enzymes are very sensitive to temperature, and it is entirely probable that the temperature change has affected the rate of enzyme activity directly, thus changing the speed of one or more links in the chain of reactions. On the other hand, a number of modern workers incline to the view that protoplasm has a definite structure, and that the reactions proceed at the interphase boundaries. LILLIE (15) states:

Since the formation of films is a characteristic feature of the processes at phase boundaries in polyphasic systems of all kinds, and especially in protoplasm, it is probable that films are present at all sharply defined boundary

surfaces in the living cell, i.e., not only at the general surfaces of the cell and of cell structures like nuclei and vacuoles, but also at the surfaces of fibrils, granules, chromosomes, mitochondria, the Golgi apparatus, and other structures. Variations in the physical or chemical properties of these films will influence the catalytic and other properties of the surfaces, and hence the nature and rate of the chemical reactions occurring in the cell under the surface influence. Electrical factors are of special importance in all such effects; . . . . Since these films are readily broken down and reformed under often slight changes of condition, the state of the protoplasmic surface layers is subject to continual change; the rate and character of the chemical reactions occurring under the influence of these surfaces are affected correspondingly. There are many indications (apart from the action of narcotics) that the special sensitivity of the protoplasmic system to chemical, electrical, and other changes of condition, i.e., its irritability, is to be referred chiefly to this variability of the protoplasmic films.

It seems clear that any change in the rate of one of the steps in the chain of linked reactions, as the result of a stimulus, would influence the entire chain and consequently result in a changed amount of carbon dioxide being released. This explanation will satisfactorily explain the change in respiratory rate upon a change in temperature, but it will not explain the constant fluctuations in the respiratory rate just noted. The continual breaking down and building up of protoplasmic films, with the resulting changes in surface action, would seem to offer a more probable explanation for these fluctuations. Probably both of these processes are concerned. Protoplasm undoubtedly has a definite structure, for the mere presence of the chemical and physical components usually found in the cell does not produce a living cell. Further, when the structure of the cell is destroyed, profound physical and chemical changes at once appear. On the other hand, many of the chemical reactions proceeding within the cell have already been established. Thus both explanations have a sound basis in fact.

Summary

1. A definite stimulation of the respiratory rate of the shoot is shown to occur as a result of temperature change of the root.

2. The amount and type of stimulation vary with the temperature at which the plants are held and the direction of the temperature change.

3. The magnitude of the stimulation does not appear to be defi-

nitely related to the size of the difference between the two temperatures used.

4. The 25° C. constant temperature series shows smaller fluctuations from its mean respiratory rate than does either the 15° or the 35° C. constant temperature series.

5. A study of the temperature coefficients shows that a marked depression in the respiratory rate occurred at 35° C., probably due to a "time factor."

6. A new type of aspirator and gas collection apparatus is described.

The writer wishes to acknowledge his indebtedness to Professor CHARLES F. HOTTES, under whose direction the investigation was undertaken, for his aid in the construction of apparatus and for his most helpful criticism and suggestions during the progress of the work.

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EFFECT OF MANGANESE, COPPER, AND ZINC ON GROWTH AND METABOLISM OF ASPERGILLUS FLAVUS AND RHIZOPUS NIGRICANS[1]

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(WITH SEVEN FIGURES)

Fungi absorb mineral nutrients, such as manganese, copper, or zinc, from the soil or from solutions, but not possessing photosynthetic metabolism, they are dependent upon plant or animal products for their food. In the natural growth of fungi, therefore, manganese, copper, and zinc in small amounts are present in the medium on which they grow. In a synthetic medium such as the average laboratory culture, these elements usually are present as impurities in sufficient quantities for the normal growth of plants.

The findings of previous investigators, although somewhat inconsistent, seem to indicate that manganese, copper, and zinc are necessary for the normal growth of such species of fungi as have been investigated. SAUTON (5) concluded from his experiments that manganese was as indispensable to the spore formation of *Aspergillus fumigatus* as was oxygen. WATERMAN (8) found that manganese increased the rapidity of spore formation. He also investigated the effect of copper and zinc, finding that certain concentrations of zinc sulphate, zinc chloride, and copper sulphate increased the weight of his cultures but decreased spore formation proportionately. WATERMAN considered the influence of copper and zinc unfavorable. Zinc was found by LEPIERRE (3) to exert, under certain conditions, a favorable influence on the growth of *A. niger*, but not to be absolutely essential for complete development. JAVILLIER (2) observed that zinc in a concentration of one part to two million parts of medium increased the volume of growth of *A. niger* 58 times. Further work

[1] Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station. The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

by JAVILLIER showed that zinc could not be replaced by cadmium or beryllium. STEINBERG (6) obtained a minimum growth with *A. niger* in the absence of iron and zinc, and regarded both metals as essential for normal growth. Later experiments by STEINBERG indicated that iron and zinc could partly be replaced by uranium or cobalt. Metallic zinc was found by BORNAND (1) to decrease the growth of *A. niger*. ROBERG (4) studied the effect of pure compounds of iron, copper, and zinc on the growth of representative species of *Aspergillus*. Iron and zinc were shown to be essential for growth but the results obtained with copper were not decisive. The writers believe the indecisive result obtained with copper was due to his inability to exclude copper contamination in the nutrient solution.

To ascertain the effects of manganese, copper, and zinc on the growth and metabolism of molds, it was necessary to prepare a medium favorable for their development but free from these elements. A solution containing 1 per cent ammonium sulphate, 0.5 per cent monopotassium phosphate, 0.4 per cent potassium sulphate, 0.25 per cent magnesium sulphate, 0.25 per cent calcium sulphate, and 5 per cent glucose in distilled water produced a heavy, rapid growth of several molds. A standard medium of this composition, using sucrose instead of glucose (because the latter contained impurities) was then prepared, employing only salts that had been shown to be free from manganese, copper, and zinc by chemical tests. The distilled water used in all the cultures was prepared by means of a quartz tube condenser. In purifying these salts from manganese, copper, and zinc, other elements usually present as impurities were also removed. To avoid any influence due to the absence of these elements, 0.001 per cent ferric citrate, 0.0001 per cent potassium iodide, and 0.01 per cent potassium chloride were included in the medium. Agar could not be obtained free from manganese, zinc, and copper, so silica gel was added to the liquid medium for solid cultures.

Growth of molds on this medium was very weak and slow. A green *Aspergillus* of the *flavus* group made the most vigorous growth, and was isolated in pure culture. Six transfers of spores were made on the standard medium to prevent any appreciable amount of manganese, copper, and zinc being carried over in the inoculum, before

the experimental culture was accepted. Cultures were incubated at different temperatures, and optimum growth was obtained at 28° C.

Cultures were then started in three series of flasks, using 100 cc. of medium in each. Manganese sulphate was included in the medium in the flasks of the first series in such quantities that the manganese content of each flask differed five parts per million from that of each adjacent flask. The range in concentration covered by the series was from a blank control (0) to 30 parts per million. Copper sulphate in corresponding amounts was added to the second series of flasks, and in the third series zinc sulphate was varied by five parts of zinc per million from a blank to 30 parts per million. Each of the salts of manganese, copper, and zinc added to the medium was known to be free from the other two elements. Growth was rapid in some flasks, a maximum being reached in five days. Cultures were then filtered and the fungi washed with distilled water to remove adhering medium. An excess of water was used over the amount found necessary to remove sulphates, and the same quantity was used in washing each culture. The weight of each culture was recorded after drying at 100° C. to constant weight, and compared with the controls and with each other as the amounts produced in 100 cc. of medium in five days.

All cultures containing manganese, copper, or zinc produced a greater weight than the controls (fig. 1). The greatest weight in the manganese series was produced by the culture containing five parts per million of this element. A nearly uniform decrease in weight occurred with each addition of manganese over this concentration, up to 25 parts per million. Thirty parts per million produced a slightly greater decrease than 25 parts per million. The conidia were a yellowish green when manganese was present, and a pale green to white in its absence. At 20 parts per million manganese appeared to be toxic to the *Aspergillus*, and conidiophores were not produced abundantly, none being produced near the edges of the colony. Twenty-five parts per million and greater concentrations were decidedly toxic, the mycelium being curled and conidiophore production greatly retarded. The optimum concentration of manganese sulphate was below five parts per million.

The series of cultures containing copper also produced a maximum

growth at a concentration of five parts per million, and lost weight with each increase in the copper content. The increase in weight with copper was not quite so great as that produced by manganese. Copper sulphate was toxic in concentration exceeding 15 parts per million. The spores of all cultures grown in the presence of copper had a uniform blue-green color. Zinc salts were toxic at a concentration of five parts per million. Conidia were not so abundant as in the
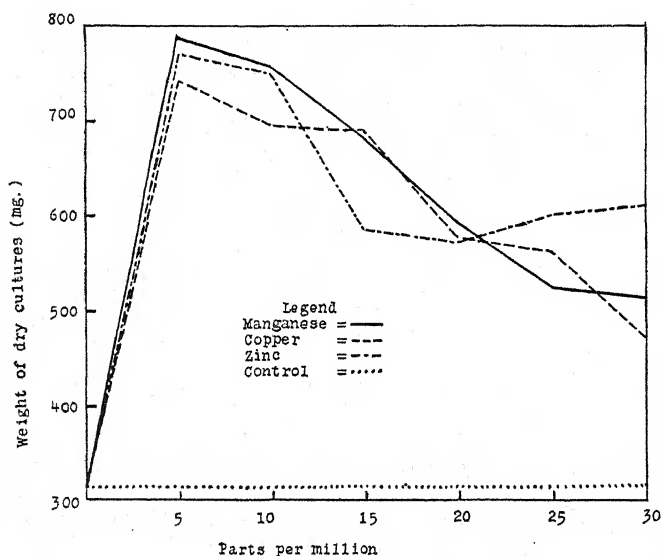

FIG. 1.—Amounts of growth produced in cultures containing varying amounts of manganese, copper, and zinc.

series containing manganese or copper. The colonies were wrinkled and curled, and marked by white edges and light zones. The greatest weight was produced in the presence of five parts per million of zinc. The weight of cultures decreased with increases in the concentration of zinc to 20 parts per million. Greater concentrations gave inconsistent results. The maximum weight produced in the zinc series was slightly greater than that of the copper series, but was exceeded by the maximum weight obtained in the manganese series.

The optimum concentrations of these salts were investigated on silica gel cultures, since it was evident that they were a factor in the

growth of the *Aspergillus*. These were found to be 5 parts per million for copper, 2.5 parts per million for manganese, and 1 part per million for zinc (fig. 2). Copper was also found to be more toxic when either
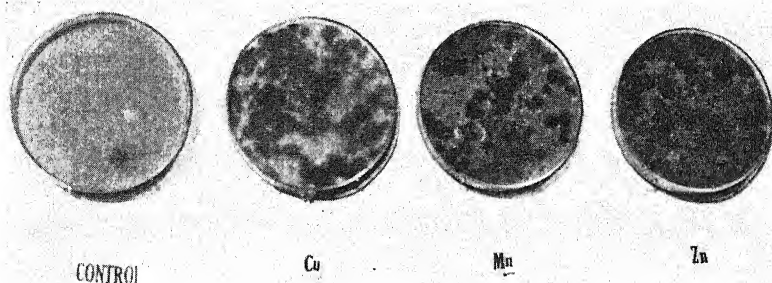

CONTROL   Cu   Mn   Zn

FIG. 2.—Sand cultures of *Aspergillus* 10 days old, showing stimulation of copper (5 ppm), manganese (2.5 ppm), and zinc (1 ppm) in optimum concentrations on growth.

manganese or zinc was present. The influence of the optimum concentrations of these metals was ascertained in fluid cultures of 250 cc. each. Manganese, copper, and zinc, and all combinations of these elements in the optimum concentrations were represented, otherwise the chemical composition of the cultures was identical. Such factors as length of time and temperature of sterilization, hydrogen-ion concentration, and temperature and period of incubation were the same for all cultures. The amount

FIG. 3.—Effect of manganese and zinc added to copper.

of spores used for inoculation probably varied in different cultures. Because of an oil which covered the spore, a suspension could not
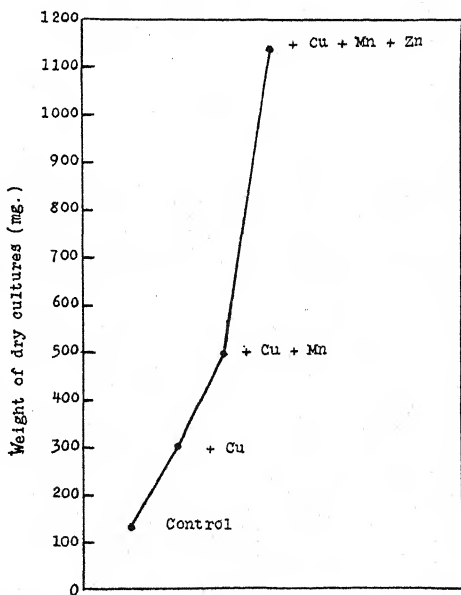
be formed, so the smallest amount of material that could be handled on a pointed needle was transferred to each flask. All cultures were incubated at 28° for 5 days.

After attaining the optimum growth, the cultures were filtered, washed free from the nutrient solution, dried at 100 °C. to constant weight, and the weig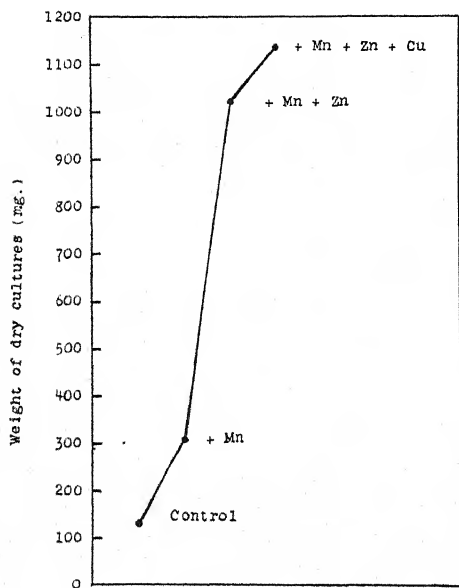hts determined and compared. Manganese and zinc influenced growth to a greater extent than did copper. In combination also they produced a greater effect than either metal in combination with copper. Fig. 3 shows the increase produced in total weight by copper over the control. The addition of manganese in the culture resulted in a greater increase, while the most rapid growth occurred when all three of these elements were in the culture. Manganese (fig. 4) produced an increase slightly greater than did copper. Manganese and zinc increased the weight to a much greater extent than did manganese and copper, or zinc and copper (fig. 5). Zinc with copper influenced growth to a greater extent than manganese and copper. The relation between these cultures, in terms of the control as 100 per cent, was:

FIG. 4.—Effect of zinc and copper added to manganese.

CULTURE	PER CENT
Control	100.0
Copper	234.5
Zinc	241.0
Manganese	247.5
Copper+manganese	399.7
Copper+zinc	510.8
Manganese+zinc	857.9
Copper+manganese+zinc	900.6

The size of the colonies on silica gel plates compared closely with the weights of the dried mold from the liquid cultures. All plates containing copper, manganese, or zinc produced colonies of greater diameter than did the control plates. The growth was measured daily and recorded (fig. 6). The cultures were also photographed. Because of the greater contrast of the colonies (fig. 7), *Rhizopus nigricans* showed the effects of copper, manganese, and zinc more clearly when photographed than did the *Aspergillus*.

The smaller volume of medium and the less rapid removal of catabolic products resulted in slower growth on silica gel for all cultures, as compared with that in liquid media. This effect was more pronounced on cultures making a large amount of growth. The control culture (fig. 6) made most of its growth between the first and third day after inoculation, and ceased growing on the fifth day. When manganese, copper, or zinc was present, a greater and more rapid

FIG. 5.—Effect of copper and manganese added to zinc.

growth was obtained, the growth curves for these elements corresponding closely to one another. Combinations of two of these elements also formed curves that were closer to one another than to the control curve, or to the curves of the elements when but one was present, or when all three were present. The sudden stopping of growth in the control culture indicated a deficiency of necessary nutrient elements rather than toxic inhibition, in view of the much greater and more rapid growth of the other cultures. This is also shown to a less marked extent by all cultures not having all three elements present. The decrease in the rate of growth of cultures con-
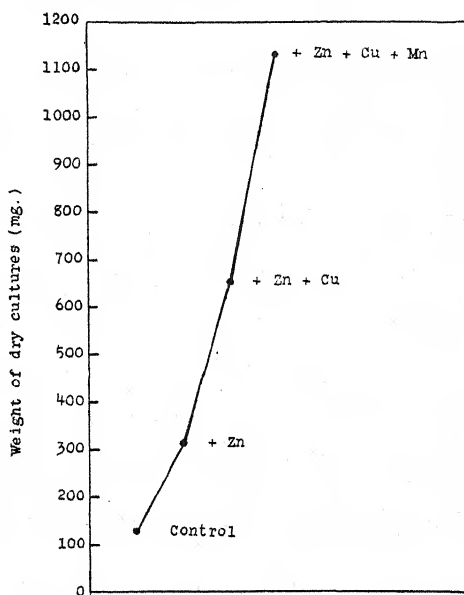
taining copper, manganese, and zinc might be due to the absence of other elements necessary for growth, or to toxic accumulations.
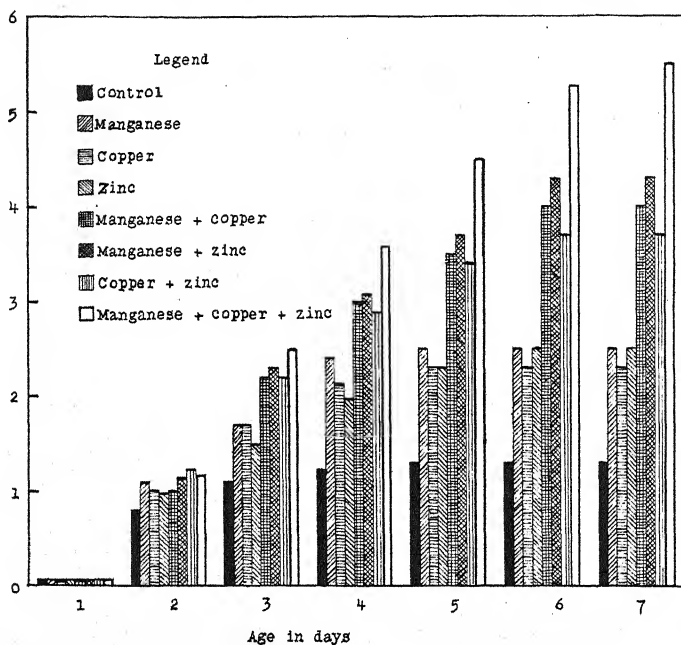

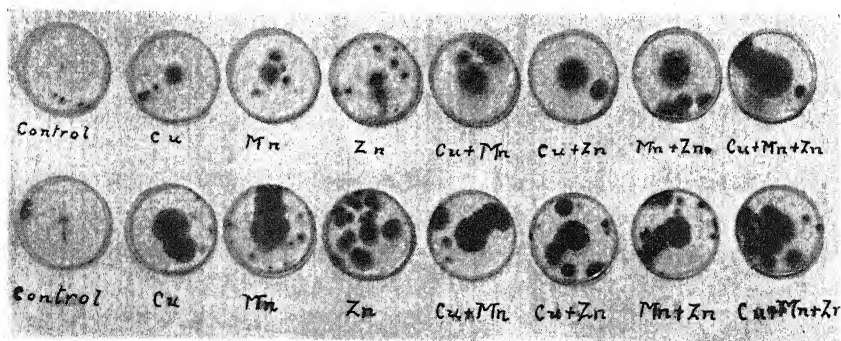FIG. 6.—Effect of manganese, copper, and zinc on cultures grown on silica gel

FIG. 7.—Silica gel cultures of *Rhizopus nigricans* (upper row) and *Aspergillus* (lower row), fifth day of growth, showing influence of manganese, copper, and zinc, and their combinations on growth.

The presence of copper in the media apparently slightly increased the plants' ability to assimilate other mineral nutrients; manganese produced the opposite effect. The cultures grown on media containing manganese had lower percentages of ash than the control unless zinc was also present. All cultures containing zinc had a higher ash content than the control.

Copper was present in the ash of all cultures in greater quantities than either manganese or zinc, the percentage of copper being higher

TABLE I

MINERAL, NITROGEN, AND FAT CONTENT OF ASPERGILLUS IN
PERCENTAGE OF MOISTURE-FREE MATERIAL

CULTURE	CONTROL	Cu 5 PPM	Mn 2.5 PPM	Zn 1 PPM	Cu 5, Mn 2.5 PPM	Cu 5, Zn 1 PPM	Mn 2.5, Zn 1 PPM	Cu 5, Mn 2.5, Zn 1 PPM
Weight of culture, as percentage of control.....	100	234.5	247.5	241.0	399.7	510.8	857.9	900.6
Ash............	5.0	5.29	4.75	6.03	3.98	7.67	6.046	5.5
Copper.........	None	0.017	None	None	0.013	0.022	None	0.02
Manganese.....	None	None	0.0025	None	0.0025	None	0.0023	0.003
Zinc...........	None	None	None	0.009	None	0.0076	0.0097	0.0075
Phosphorus.....	0.9	0.977	0.157	1.26	0.95	1.14	1.415	0.957
Calcium........	0.21	0.233	0.12	0.484	0.252	0.667	0.583	0.415
Magnesium.....	0.255	0.197	0.13	0.335	0.282	0.275	0.203	0.467
Iron...........	0.002	0.009	0.005	0.007	0.014	0.02	0.0076	0.008
Nitrogen........	4.82	4.75	4.65	4.5	4.09	4.25	4.4	4.48
Protein (Nx 6.25)	30.1	29.75	29.00	28.1	25.6	26.6	27.48	28.00
Ether extract....	1.64	2.03	2.55	2.25	2.51	2.57	3.355	3.5

in the presence of zinc and lower in the presence of manganese. The amounts of manganese in the ash varied but slightly in the presence of the other two metals. The zinc content was increased by manganese and reduced by copper.

Phosphorus, calcium, and magnesium were assimilated in larger quantities by cultures containing zinc. The absorption of these elements was lowered by the presence of manganese unless copper or zinc was present. Less magnesium was absorbed by the copper culture than by the control. The ether extract increased as growth increased, the increase being greater when manganese was present. Zinc was next to manganese in influencing the synthesis of the ether-soluble products.

The nitrogen content was less than the control in all cultures containing manganese, copper, and zinc. The percentages of nitrogen did not appear to be decreased by any one element, or uniformly decreased by an increase in growth. Apparently nitrogen decreased as growth increased, unless manganese and zinc were present. Analyses are given in table I.

No consistent differences could be found by microscopical measurements of the morphological structures of these cultures. The sizes varied in all cultures within the limits described by THOM and CHURCH (7) for the species.

Summary

1. Cultures of *Aspergillus* made a heavier, more rapid growth in a medium containing certain concentrations of manganese, copper, or zinc than in a medium free from these metals.

2. The optimum concentrations of these metals were low, and slightly larger quantities became toxic.

3. Combinations of the optimum concentrations of manganese, copper, and zinc stimulated a greater growth than did any one of the three metals, and all three produced a greater growth than did the combination of any two of these metals.

4. Manganese, copper, and zinc in the medium influenced slightly the assimilation of phosphorus, magnesium, and calcium.

5. The nitrogen content of *Aspergillus* grown in media containing manganese, copper, and zinc, or combinations of these elements, was less than in control cultures.

6. The fat content (ether extract) of *Aspergillus* was increased by manganese, copper, and zinc.

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NUCLEAR DIVISION AND DEVELOPMENT OF STERIGMATA IN COPRINUS ATRAMENTARIUS

MARGARET MARTIN VOKES

(WITH FORTY-EIGHT FIGURES)

Introduction

The hymenia of the Basidiomycetes have been investigated by HOFFMAN (14), DE BARY (8), ATKINSON (1–3), and others. NICHOLS (23) and LEVINE (19) germinated individual spores in order to study the mycelia and sporophores at will. Cytologically, however, little has been done with this group, and this study was undertaken to consider that phase.

In 1884 STRASBURGER (28) studied the division of nuclei in the basidia of the Basidiomycetes. He stated that the nucleus divided into as many as eight, but that formation of the sterigmata began when the nucleus within the basidium had divided into two. He was certain centrospheres were present, either inside or outside the nucleus. In 1866 DE BARY (8) had observed a clear central portion in the basidia of the Agaricaceae, which he assumed to be a nucleus but which he said needed further study. ROSENVINGE (26), working with STRASBURGER, concluded that all the cells of the Hymenomycetes contained one or more nuclei. In the young basidia the single nucleus divided, forming four or eight nuclei, which, together with the protoplasm of the basidium, pushed their way up through the narrow neck of the sterigmata and into the spores. The number of nuclei was constant for the same species but not always for the genus.

Later WAGER (30–33) investigated the Hymenomycetes cytologically, chiefly *Agaricus stercorarius* and *Amanita muscaria*. He found the young basidia to contain two nuclei, together with a small amount of protoplasm and one or two vacuoles. At an early stage these nuclei fused, forming one large nucleus at the center of the basidium. This nucleus later took up a position near the apex of the

basidium, where during division a spindle appeared, and at each pole a deeply staining granule or centrosome was present. The resulting nuclei again divided. The nucleolus apparently played an important part during division, being found at the upper end of the nucleus. The daughter nuclei were similar to the parent nucleus, but smaller. Previously or during formation of the sterigmata the four nuclei became closely grouped at the base of the basidium, appearing almost fused. Spores protruded from the tips of the sterigmata, and protoplasm from the basidium passed into the spores. Not until then did the nuclei pass to the apex of the basidium, becoming so small that they could pass without difficulty into the spores, but whether such passage took place was not determined. WAGER (31) agreed with ROSEN (25), who studied *Lepiota (Armillaria) mucida*, that a much coiled thread was present within the network of the single nucleus.

DANGEARD (4–7) reviewed previous work, and after studying a number of species, concluded that all cells contained two nuclei, each having two chromosomes, and that these nuclei fused to form a single large nucleus in the basidium. Such fusion constituted a gametic union.

RUHLAND (27) investigated several species and found spindle rays as well as centrosomes and astral rays. HARPER (13) found spindles in *Hypochnus subtilis* on which were 6–8 chromosomes. That the binucleate condition existed in all cells of the sporophore was again definitely concluded. PETRI (24) observed, in *Hydnangium carneum*, strands which he considered extensions of the nuclear membrane and which connected the nucleus and the sterigmata.

One or more species in nearly every family of the Basidiomycetes was studied by MAIRE (20–22), and in all of them two nuclei only were observed to be associated with mitotic conjugation in the young basidia. The two nuclei fused, the chromosomes became thick, divided in two longitudinally, were torn apart, and went to the poles where astral rays were then emitted. The two nuclei formed produced spindles almost immediately, each having centrosomes and asters. Later four centrosomes occupied the extremities, and seemed to determine the location of the four spores. Before each centrosome went toward the apex of the basidium, a kinoplastic differentiation

was produced in the cytoplasm of the basidium; under the influence of this the nuclei were placed in a line along the axis of the basidium, and little by little were attracted toward the summit of the cell. The extremities of the sterigmata then swelled, and all the cytoplasm passed into the spores whose membranes had begun to enlarge at the time the nuclei reached the entrance of the sterigmata below. Two chromosomes were thought to be present in all species.

VAN BAMBEKE (29) reported fibers which connected nuclei and centrosomes in the basidia of *Hydnangium carneum*. Later FRIES (10) reported the same for *Nidularia pisiformis*. He found centrosomes and astral rays on the primary spindle, and longitudinal splitting of the spindle was thought to occur. GILBERT (11, 12) found centrosomes in *Dacryomyces* sp., and a dense staining body on the nuclear membrane during late synapsis which he believed gave rise to four centrosomes.

LEVINE (17–19), studying chiefly *Boletus* and *Polyporus*, found the two basidial nuclei fused. Spireme strands suggested splitting, and a shortening and segmentation of chromatin followed. The orientations of the spindle axes varied but were commonly transverse to the long axis. The polar asters of LEVINE (18) corresponded with those seen by MAIRE (22). The four basidial nuclei did not at any time go to the base of the cell and fuse, as WAGER had reported, but remained in the central position. At this time a spore initial in the form of a globular mass of cytoplasm became differentiated at the apex of each sterigma. A fibrillar strand was stretched from each centrosome at the apex of the sterigma to the nucleus, which was still in the basidium. This had been reported by MAIRE (22), who thought this strand contracted and pulled the nucleus up into the spore. In the species of *Boletus* studied there were 6–8 chromosomes, and although the number in the second division could not be determined, it was always more than two.

Eocronartium muscicola was studied by FITZPATRICK (9). His plates show spindles but do not show centrosomes. KUHNER (15, 16) found both centrosomes and spindles, and although division of the conjugate nucleus was thought to have taken place transversely, it usually occurred at the apex of the basidium.

Materials and methods

Several sporophores of *Coprinus atramentarius* were collected on the campus of the University of Chicago in October, 1928, by Professor C. J. CHAMBERLAIN. These sporophores were in the button stage and were cut in pieces 1–3 mm. cube and fixed in chromo-acetic-osmic solution. The paraffin ribbons were cut at 1.5 μ, and were stained with iron-alum-haematoxylin.

Investigation

Longitudinal sections of gills show loose arrangement of vegetative hyphae, the trama, through the center. The cells are binucleate
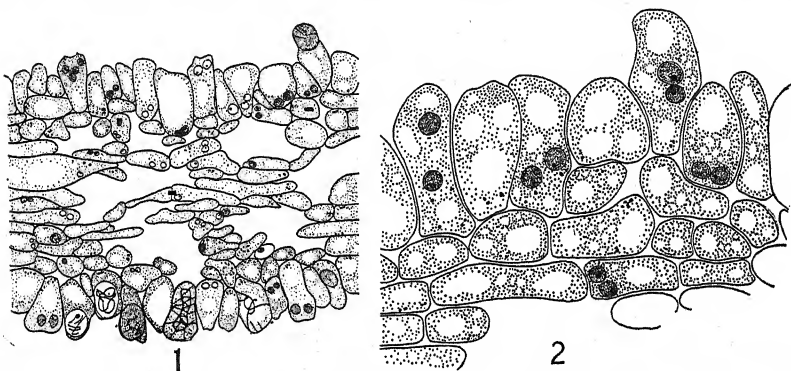
FIGS. 1, 2.*—Fig. 1, longitudinal section of young gill; fig. 2, early binucleate stage of hymenial layer.

* Figs. 1–3 were made with a Spencer fluorite objective 1.8 mm., combined with a Zeiss compensating ocular 10×. All figures were made with the aid of a Spencer camera lucida.

and hemispherical pads are present (fig. 1). Larger and slightly more compact cells, the hymenial layer, grow out from the trama, and here the basidial cells are produced which in turn produce the basidiospores (fig. 2). All have the contour of the basidia, but some are smaller and sterile (paraphyses). They are arranged more or less alternately with the spore-producing cells. Because of these paraphyses the spores are better able to expand.

Other large, well-filled cells, the cystidia, connect one gill with another. They originate, as do the hymenial cells, in the trama, but these become several times larger than the surrounding cells and

finally protrude into the opposite gill. They are club-shaped, bi-
nucleate, and contain many vacuoles. Cystidia have been the cause
for much speculation, and a wide variety of functions has been given
them (fig. 3).

Two nuclei are always present at the base of the young basidial
cell (fig. 4). The remainder of the cell contains cytoplasm and large
vacuoles which, at this time, are present in the center and near the
apex. These two primary nuclei move to the center of the cell and
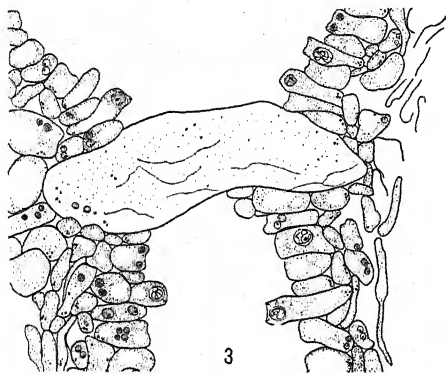fuse. At first the two nucleoli are separate in the single nucleus, but
soon merge into one (figs. 5–7). The resulting nucleus immediately swells and synapsis begins within (fig. 8).

During the prophases the chromosomes appear to be arranged in a more or less continuous spireme. At times this appears to be rather irregular (fig. 9), and the nucleolus remains distinct (fig. 10) but soon disappears. The spireme becomes threadlike and shows

FIG. 3.—Hymenial layer showing cysidia

longitudinal splitting (fig. 11). While the prophases are in progress the
nucleus is gradually migrating upward, until it reaches the apex of the
basidium, where it assumes large proportions, at times occupying the
entire space and touching the basidial wall (fig. 11). It does not
seem to stay in contact with the wall for any length of time, for it
has always withdrawn by the time the spireme within it segments
(fig. 12). This slight contact, however, permits the nuclear mem-
brane to leave tiny hyaline bodies attached in four places to the
interior margin on the wall above. These hyaline bodies appear to
be homologous with the structures which MAIRE (22) and LEVINE
(17–19) call centrosomes, although they are distinct from those
found later at the poles of the spindles. As the nucleus withdraws
from the wall it seems to pull out fine fibrillar strands from the four
hyaline bodies. These strands remain attached to the nucleus, and
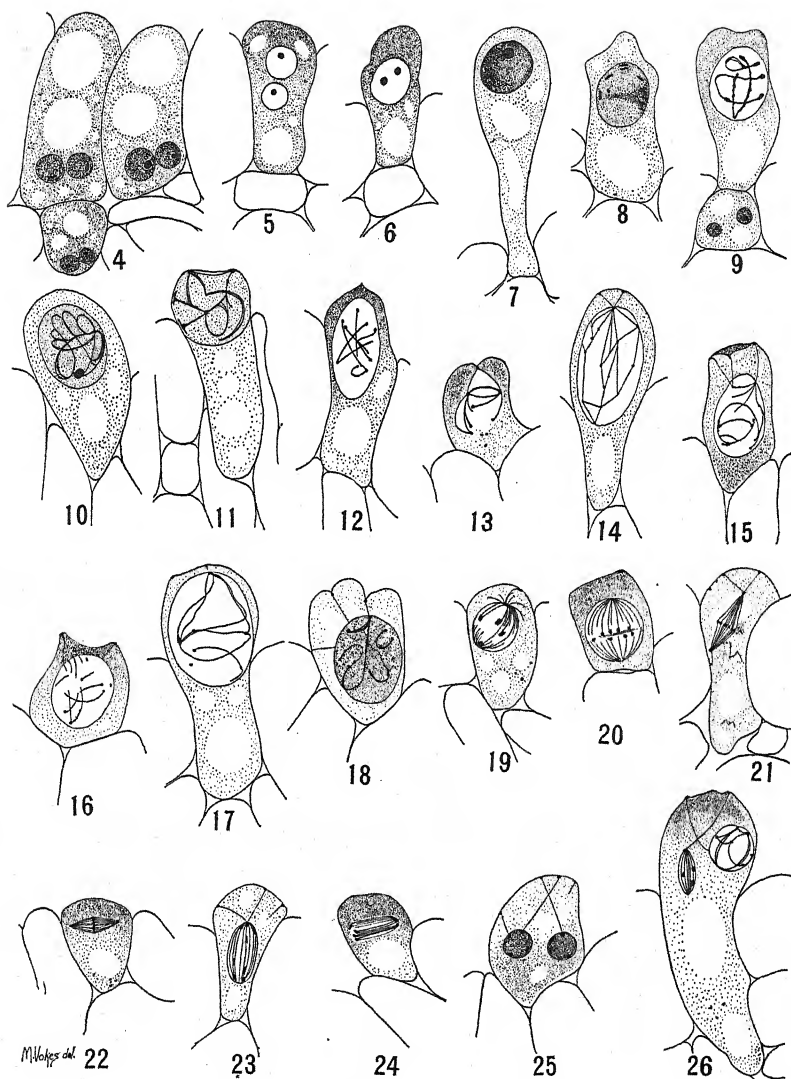
FIGS. 4–26.*—Figs. 4, 5, primary binucleate basidial cells; fig. 6, recent nuclear fusion, two nucleoli still present; fig. 7, same, nucleoli now fused; fig. 8, nucleus enlarged; synapsis beginning; figs. 9, 10, prophase, enlarged nucleus migrating to apex of basidium; fig. 11, nuclear membrane in contact with basidial wall at apex; fig. 12, nucleus moving toward center of cell; figs. 13–17, cytoplasmic strands attached to both hyaline bodies and prophase nucleus; fig. 18, basidial wall pulled in by downward movement of prophase nucleus; hyaline bodies attached firmly to wall and fibers tight; fig. 19, four chromosomes appear to be bivalents; fig. 20, disjunction of chromosomes reduction division; fig. 21, equatorial plate stage; fig. 22, position of spindle lying at different angle from that in fig. 21; fig. 23, anaphase; fig. 24, early telophase; fig. 25, two daughter nuclei; fibrillar strands remain attached, two to each nucleus; fig. 26, second nuclear division.

* Figs. 4–48 were made with Zeiss 2 mm. apochromatic objective (N.A. 1.40 and 1.30) combined with Zeiss compensating ocular 15 ✕.

later to the daughter nuclei which result from the division of the parent (figs. 11–18). It is not always possible to show four such fibrillar strands, but four hyaline bodies are certainly laid down by the single nucleus during the contact of membrane and cell wall, the fibers being pulled out by the withdrawal of the nucleus to the center of the basidium. By the time the nucleus has returned to the middle of the cell the spireme within has become short and segmented (figs. 12–16). A spindle is then formed which presents the metaphase, the chromosomes being present at the equator. Four chromosomes are seen which appear to be bivalents (fig. 19). Fig. 20 shows four chromosomes passing to each pole, and fig. 24 represents the telophase in which the chromosomes have reached the poles. Two daughter nuclei are then formed which are about the size of the nuclei in the young basidium. Each of these has cytoplasmic fibers attached to two hyaline bodies at the apex of the basidial wall. These fibrillar threads are not always straight, sometimes appearing to be loose, but more often found crossed or twisted about each other, giving the appearance of branching. They always extend, however, from the hyaline body on the wall to the nuclei below (figs. 25, 26).

The second reduction division immediately takes place (figs. 26, 27). In some instances one nucleus in a basidium will be in prophase while the other has a spindle formed (fig. 26). The nucleus when in prophase is always larger. Usually the divisions of two nuclei in one basidium are simultaneous, the four small nuclei appearing at about the same time (fig. 35). When four nuclei are present in the basidium each nucleus is attached by a fibrillar strand to a hyaline body above. Figs. 29, 32–41 show the influence of the hyaline bodies to both basidial wall and nuclei. Often, before the second division is completed, the basidial wall bulges up in four places, where a hyaline body remains within at the apex of each protrusion. By the time the four nuclei have developed great progress has been made by the sterigmata, which have protruded and the nuclei have begun their upward movement.

The hyaline bodies remain fixed to the apex of the growing spore wall, which enlarges greatly after the entrance of the nucleus. Within the spore cytoplasmic threads seem to be in a variety of positions. No distinct nucleus can be determined, but the hyaline bodies can

usually be discerned at the apex. This body, at one time so closely associated with the fusion nucleus, has remained at the apex of the basidial wall during two mitoses, goes upward within the peak of the sterigmata, and is at the apex of the young spore (figs. 11–15, 28–48). Since no definite orientation of spindles is present, a spindle will sometimes be in such a position as to block a nucleus from entering
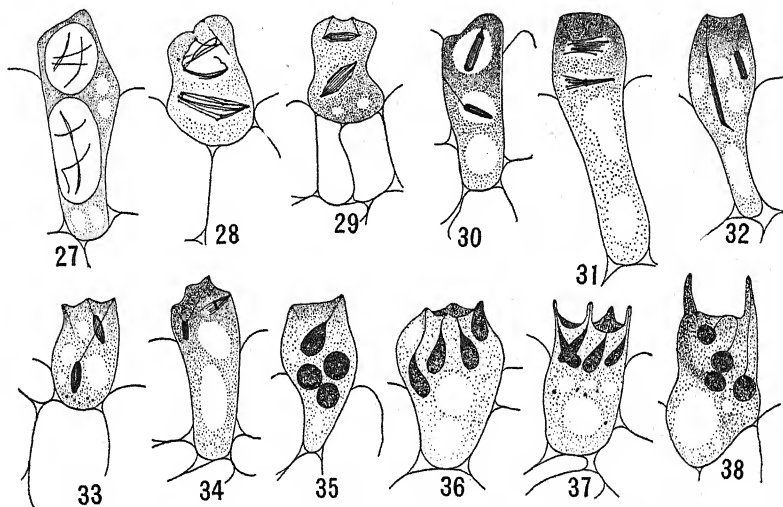

FIGS. 27–38.—Fig. 27, prophase of second division; fig. 28, spindle formation and spindle in anaphase; fig. 29, anaphase; spindle showing four chromosomes and attachment to hyaline bodies at both poles; figs. 30–32, spindles in various stages of division; fig. 33, early telophase; cell wall projects decidedly where hyaline bodies are attached; fig. 34, spindles in early telophase and equatorial plate stages, latter attached directly to hyaline body; fig. 35, four nuclei formed; fig. 36, four nuclei, each attached to a strand, migrating toward projection where hyaline body is attached; figs. 37, 38, migration of nuclei.

a sterigma. These variations probably account for the differences in the maturation of spores.

In the young basidia the two nuclei are at the base of the cell, and large vacuoles are present in the center and near the apex. After fusion and migration of the nucleus to the apex, the vacuoles change their position to the base. The cytoplasmic content of the cell is not dense. The placement of the hyaline body on the wall is important, for where it is located protrusion occurs, although at no time does

the wall appear to be thinner at that place. After the spores are formed the basidium continues to contain cytoplasm and large vacuoles. The basidial cell remains rigid and does not break down. Eight chromosomes are thought to be present in the fusion nucleus, and reduction apparently takes place in the first division.

WAGER (31), LEVINE (17–19), and KUHNER (15) agreed that the single nucleus moved to the apex of the basidium. They also ob-
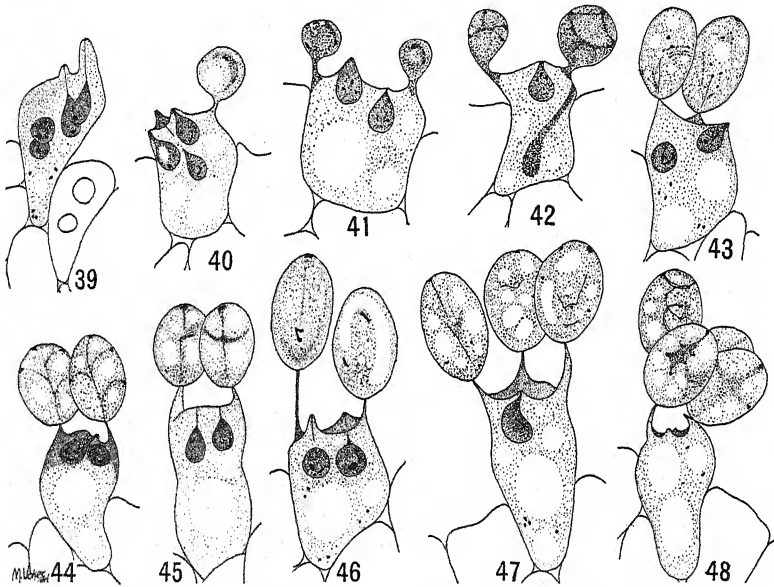
FIGS. 39–48.—Fig. 39, nuclei in various stages of migration; fig. 40, single spore in early stage; three nuclei still in basidial cell but nearing sterigmata; fig. 41, two young spores formed and two nuclei migrating; fig. 42, spore membrane enlarged after entrance of nucleus; figs. 43–46, two spores reaching maturity; two nuclei still within basidium; fig. 47, three spores formed; delayed nucleus migrating; fig. 48, four spores formed; basidial cell now devoid of nuclei.

served "centrosomes" and fibrillar threads. Fibrillar threads, MAIRE (22) thought, contracted and brought the nucleus up into the sterigma. LEVINE (19) observed these fibers, but not until four daughter nuclei were produced, after which the fibrillar strands apparently pulled the nuclei up into the spores. This hardly seems necessary, for the "centrosome" which is attached to the apex of the sterigma moves up with the growth of the spore (which is not a spore initial

but an outgrowth or protrusion of the spore membrane), and this fiber is certainly not any longer, if it is as long, as the height of the sterigma and spore combined; and the nuclear content undoubtedly is coherent. The nucleus then merely passes along with the elongation of the sterigma and spore and appears to be held by the fibrillar strand.

WAGER's phenomena regarding the homologous massing of nuclei at the base of the basidium was not apparent in this study, and was probably caused during fixation. It is quite possible that astral rays occur, as they do in some fungi, but it is also possible that what were interpreted as astral rays may have been cytoplasmic strands which broke during fixation and hung down into the cytoplasmic content from the poles of the dividing nuclei. With further study it is hoped to determine this point.

Summary

1. After fusion of the two nuclei in the basidial cell of *Coprinus atramentarius*, synapsis takes place in the single nucleus. This nucleus then migrates to the apex of the cell and its membrane comes in contact with the cell wall, where four hyaline bodies become attached. With the withdrawal of the nucleus to the center of the cell cytoplasmic threads are drawn out. These connect the hyaline bodies left on the wall to the nucleus.

2. The centrosomes of the spindles are not interfered with in the formation of the four hyaline bodies which remain on the basidial wall. After the first division, threads from four hyaline bodies are attached to the two nuclei; after the second division, threads from four hyaline bodies are attached to as many nuclei.

3. The long axis of the spindle has no definite orientation in either division.

4. The hyaline bodies remain attached to the apex of the cell wall from the time the fusion nucleus comes in contact with it until after formation of the spores. These bodies react on both the wall and the nucleus in such a way that the wall pushes up at their points of contact and sterigmata form. The nucleus then follows, and after it has entered the spore the wall greatly enlarges and assumes mature proportions.

5. After the hyaline bodies become attached they remain at all times at the apex. After a sterigma forms a hyaline body is at the tip as well as after the spore is formed.

6. There are eight chromosomes in the fusion nucleus. Reduction takes place in the first division.

I wish to express my thanks to Professor C. J. CHAMBERLAIN, who proposed this work; and to acknowledge my indebtedness to Dr. S. YAMANOUCHI for making the slides and microscopically verifying figures.

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PHOTOTROPIC "INDIFFERENCE" AND THE LIGHT-SENSITIVE SYSTEM OF PHYCOMYCES[1]

E. S. CASTLE

(WITH TWO FIGURES)

I

A state of phototropic "indifference" is said to exist in the light-sensitive sporangiophore of *Phycomyces* when a phototropic growth response fails to follow exposure of the sporangiophore to lateral illumination from one source. The phototropic response is termed positive when bending is toward the source of light; negative when bending is away from it. The existence of these two types of bending led earlier workers to regard the state of indifference as a resultant of two opposed phototropic reactions (OLTMANNS 6, BLAAUW 1). It has been shown (CASTLE 4, 5) that there is in the sporangiophore of *Phycomyces* one basic light-sensitive system which, according to the conditions of illumination and observation, determines two types of photic response: (1) a direct growth response, manifested as a brief acceleration in the rate of elongation of the sporangiophore; and (2) a phototropic response, or bending of the sort already mentioned. Evidence is here submitted to show that phototropic indifference in *Phycomyces* is due to equal stimulation and response on both sides of the sporangiophore, based on photic excitation occurring within one photosensitive system. An account of the mechanism of the reversal of phototropism, leading to negative bending, will be given elsewhere.

"Indifference" has been studied previously under conditions of continuous unilateral illumination (6). This method has the disadvantage that the sensitivity to light of sporangiophores which have been allowed to become dark adapted is known to change rapidly during illumination (TOLLENAAR and BLAAUW 7, CASTLE 3). Furthermore, changes in the pigmentary content of the sporangiophore may well be induced by long continued illumination.

[1] From the Laboratory of General Physiology, Harvard University, Cambridge.

In the following experiments indifference was studied following exposure to relatively brief flashes of light. Under these circumstances the sensitivity of the sporangiophore cannot change appreciably, the exposure times being as brief as one second or less. With sporangiophores of high sensitivity, illuminated from one side by light of 171 ft.-candles, a state of phototropic indifference was found over a range of exposure extending from continuous illumination down to a duration of exposure of about 0.6 second. When the exposure is further shortened, positive phototropic bending occurs.

If a sporangiophore which exhibits phototropic indifference is exposed to lateral illumination under the same circumstances, it is found that a distinct direct growth response takes place. Stimulation followed by phototropic indifference is therefore not characterized by the absence of photic excitation, but by the absence of a differential growth response.

II

The apparatus used in studying the occurrence of indifference permitted optional observation of either the direct growth response or the phototropic response, and has already been described in detail (5). It consisted essentially of a moist chamber with plate glass walls and cover held at constant temperature ($24.5 \pm 0.1^\circ$ C.) in a water thermostat, the whole inclosed by a small darkroom. Observation of a particular sporangiophore in a culture placed in the chamber was made from outside the darkroom by means of a horizontal microscope having an ocular scale. This scale could be rotated, and permitted measurement of either vertical growth or horizontal bending.

The orienting and adapting light, giving an illumination of 86 ft.-candles, was a 100-watt bulb directly above the observation chamber. Sporangiophores were brought to a state of uniform high sensitivity by thorough adaptation to this light, followed by dark adaptation of 30 minutes' duration. Dark adaptation has been shown to take place nearly to completion within this time (3).

The stimulating illumination came from a 1000-watt lamp in a special housing above the observation cell. It was reflected horizontally on to the sporangiophore by means of a 45° mirror, after passing through a heat screen and a calibrated photographic shutter

arranged to grade the duration of the exposure. The intensity of this light at the sporangiophore was 171 ft.-candles.

Readings of the position of the sporangium on the ocular scale were made at 15-second intervals against a faint red observation light. Plottings of the readings were made against time. The moment of response was taken as the first point significantly deviating from the pre-existing direction of growth, and the reaction time $(R.T.)$ as the interval between the beginning of stimulation and the moment of response. Pure cultures of *Phycomyces blakesleeanus* ($+$ strain) were used throughout the experiments.

III

The phototropic response of the sporangiophore of *Phycomyces* has been shown to occur practically simultaneously with the direct growth acceleration (4). In these experiments, therefore, the significant presence or absence of phototropic bending is noted at and near the time when photic response of the sporangiophore is known to take place. Bending occurring much later than this time is not an immediate sequel of the primary photic excitation, and does not indicate the absence of primary "indifference."

It was found that with a duration of exposure greater than 0.6 second to unilateral illumination of 171 ft.-candles, no primary phototropic response of the sporangiophore occurred. A state of phototropic indifference therefore existed. With an exposure of 0.6 second, irregular and occasional bending responses were obtained, as shown in detail in fig. 1. As the exposure time is further shortened, the occurrence of the phototropic response becomes invariable. Its regularity for different brief durations of exposure is made clear by the frequency polygons of fig. 1.

Table I gives the mean reaction times of the phototropic response with varied durations of exposure. These figures are plotted in fig. 2. The significance of the form of the curve, in showing that the velocity of the principal process taking place during the latent period is directly proportional to the amount of preceding photochemical change, has already been discussed (5). It has also been shown that the comparable curve for the direct growth response is superimposable upon the curve of fig. 2. This finding is taken to indicate

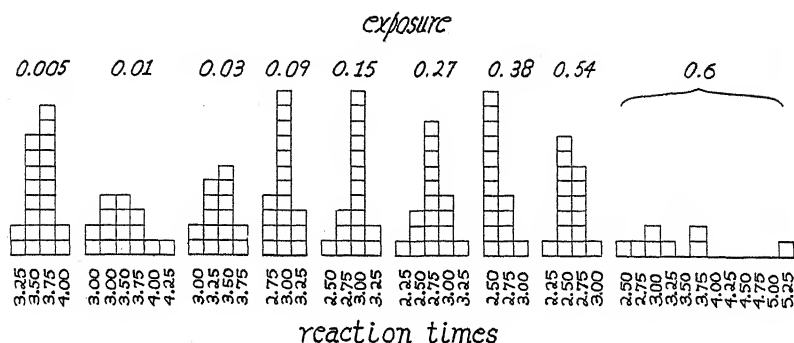that both the direct and the phototropic modes of response are based on the same photosensitive system.

FIG. 1.—Frequency polygons showing actual distributions of individual determinations of phototropic reaction times for different durations of exposure to stimulating illumination (171 ft.-candles); exposures given in seconds above each polygon; reaction time classes indicated on abscissae; each square stands on ordinate scale for one determination of reaction time.

The reaction time for the direct growth response with exposures greater than about 0.6 second is approximately constant. Below that critical duration of exposure the reaction time lengthens progressive-

TABLE I

MEAN REACTION TIMES OF PHOTOTROPIC GROWTH RESPONSE
TO VARIOUS DURATIONS OF EXPOSURE TO UNILATERAL
ILLUMINATION OF 171 FT.-CANDLES

EXPOSURE (SECONDS)	MEAN R.T. (MINUTES)*	PROBABLE ERROR OF MEAN R.T.
0.005	3.63	±0.028
0.01	3.50	0.061
0.03	3.38	0.040
0.09	2.98	0.024
0.15	2.96	0.030
0.27	2.76	0.037
0.38	2.60	0.048
0.54	2.61	0.028

* Each mean R.T. represents average of 15–22 determinations on individual sporangiophores (such averaging of photic responses is justifiable in spite of the variability in absolute rate of growth, 2, 3).

ly as the exposure shortens. Considering the processes involved in the response to light as necessarily a catenary sequence initiated by

the photochemical action of light, it is clear that with such brief exposures not enough photochemical change takes place to cause the subsequent latent period processes to proceed at a maximal rate. For the direct growth response, then, a duration of exposure of 0.6 second to light of 171 ft.-candles is critical: above that value the latent period processes involved in the response proceed at a con-
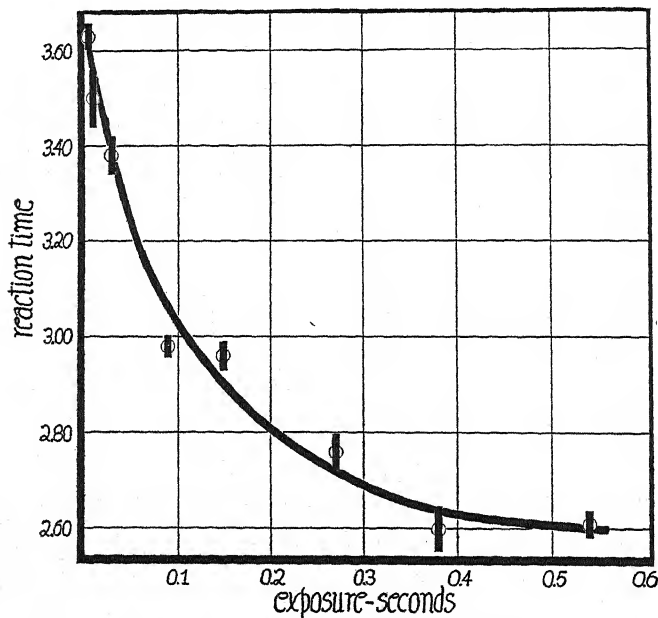

FIG. 2.—Mean reaction times (in minutes) of phototropic response plotted against duration of exposure to stimulating illumination; height of vertical bar through each symbol represents twice probable error of mean; curve is arbitrary, to show regular decrease in reaction time with increasing duration of exposure.

stant maximal rate; below that value such secondary changes are retarded in inverse proportion to the duration of the exposure (4).

For the phototropic response, also, a duration of exposure of 0.6 second is critical, in that it is the dividing line between the presence and absence of phototropic indifference, an inverse corollary of bending. For briefer durations of exposure the photochemical action of the light is evidently submaximal, at least on one side of the spor-

angiophore. Unequal action of light on the two sides must initiate secondary changes which proceed at different rates, and specifically the latent period processes on one side will advance compared with those on the other.

The observed result, bending, is thus a differential growth response, produced in this instance because the amount of light admitted to the sporangiophore was reduced until the response on one side was retarded relative to that on the other. A slight relative retardation might be expected to lead to a brief inflection of the sporangiophore, followed by an early abolition of the differential effect. Exactly such brief bendings are found with durations of exposure close below 0.6 second.

Summary

1. Sporangiophores of *Phycomyces* of high sensitivity to light, stimulated by exposure to unilateral illumination of sufficiently high intensity, fail to give the usual phototropic response. This condition is known as phototropic "indifference."

2. Sporangiophores which exhibit such indifference are nevertheless shown to give a distinct direct growth response as a consequence of the same unilateral illumination. The state of indifference is therefore not characterized by the absence of photic excitation, but by the failure of the light to evoke a differential acceleration of growth on the two sides of the sporangiophore.

3. Stimulation of sensitive "indifferent" sporangiophores by flashes of light of progressively reduced duration of exposure leads to the discovery of a critical duration below which indifference is abolished and phototropic bending occurs.

4. The critical duration of exposure to light for the appearance of the phototropic response corresponds to the critical duration of exposure for minimum reaction time in the direct growth response.

5. Phototropic bending therefore appears when the action of light on one side of the sporangiophore is submaximal. Conversely, indifference is due to equal and maximal photochemical action on both sides.

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SOLUBILITY CHANGES OF INORGANIC CONSTITUENTS IN CITRUS CUTTINGS[1]

F. F. HALMA AND A. R. C. HAAS

(WITH TWO FIGURES)

Introduction

In the propagation of citrus trees by cuttings, it has been found that the presence of leaves on the cuttings is essential to successful propagation in some species.[2] This is clearly evident from the fact that detached leaves root readily, while stems without leaves do not ordinarily respond in this way. With hardwood cuttings the stem contains the carbohydrates needed for root development; with citrus cuttings, on the other hand, the carbohydrates are supplied by the leaves, either from that stored up or that currently manufactured. Although considerable attention has been given to the importance of carbohydrate changes during the rooting process of cuttings, yet to the writers' knowledge no data are available as to whether there are any concurrent changes in the solubility of inorganic constituents. It was thought desirable, therefore, to determine what solubility changes, if any, take place in the inorganic constitution.

This investigation was purposely confined to the study of solubility changes, rather than to that of the actual amount of translocation, for the reason that in the latter case it would be extremely difficult to determine the amounts involved in material of this kind. With older stem cuttings, having a correspondingly larger root system, this might be possible were it not for the fact that the continuous development of vegetative growth introduces a new factor.

Citrus cuttings, for several reasons, are well adapted to a study of solubility changes of inorganic constituents. Cuttings can be grown successfully at any time of the year, and if of similar age and

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[1] Paper no. 219, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] HALMA, F. F., Propagating citrus by cuttings. Calif. Citrograph 11:225. 1926.

taken from the same tree, rather uniform plants can be obtained. Furthermore single leaves may be rooted, thus eliminating the stem as a complicating factor. Generally such cuttings, when grown under suitable conditions in washed sand, produce a well developed root system within three months. Normal twig growth occurs only after the rooted cuttings are transferred to a nutrient medium.
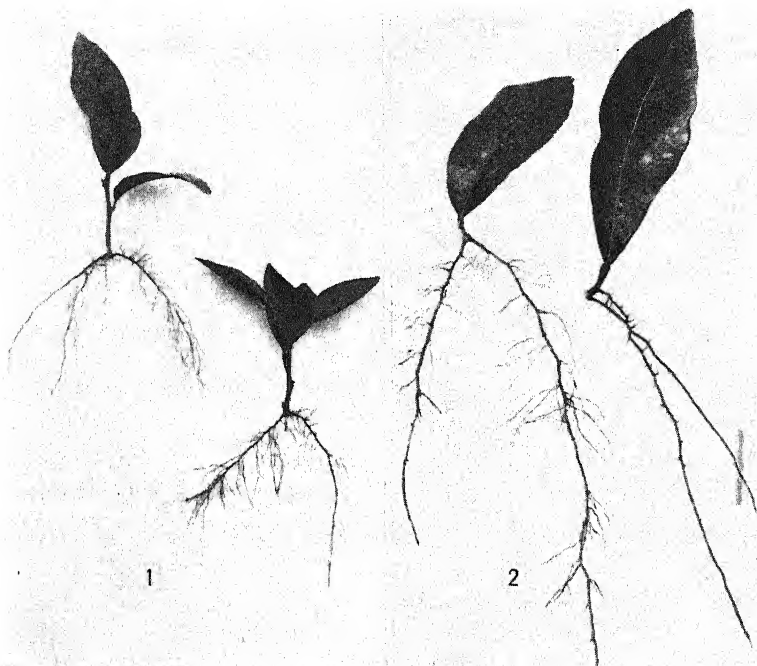
FIGS. 1, 2.—Fig. 1, rooted stem cutting of Eureka lemon and Navel orange; fig. 2, rooted leaf cutting of Eureka lemon and Navel orange.

Cuttings in sand

Eureka lemon (a variety of *Citrus limonia* Osbeck) and Navel orange (a variety of *C. sinensis* Osbeck) constituted the material for this study. One hundred stem cuttings (fig. 1) and 100 leaf cuttings (fig. 2) of each species were made. Three healthy mature leaves were left attached on each stem cutting. Fifty of each lot were planted in washed sand in a propagation frame and the other fifty were prepared for analysis in the following manner. In the stem cuttings the

apical, middle, and basal leaves, as well as the stems, were analyzed separately. Similarly the 50 leaf cuttings constituted a separate sample. The cuttings rooted satisfactorily in the propagation frame, and after three months they were taken out and prepared for analysis. Unpublished data by HALMA show that there is a high positive correlation between total leaf area or total green weight of leaves and total amount of roots produced, hence it is obvious that the single leaf cuttings produced less roots than the 3-leaved stem cuttings.

TABLE I

SOLUBLE CALCIUM, POTASSIUM, AND MAGNESIUM AS PERCENTAGES
OF WATER-SOLUBLE ASH IN THE DRY MATTER

MATERIAL	CALCIUM		POTASSIUM		MAGNESIUM	
	Control	Rooted	Control	Rooted	Control	Rooted
50 Eureka lemon stem cuttings:						
Apical leaves..................	25.84	26.38	14.55	9.02	8.24	7.95
Middle leaves................	24.82	25.95	13.91	11.15	7.75	8.20
Basal leaves.................	23.96	24.71	13.68	10.94	8.05	7.80
Stems.......................	23.60	14.77	20.95	18.39	10.90	7.52
50 Eureka lemon leaf cuttings....	25.87	23.75	12.14	12.05	5.18	6.08
50 Navel orange stem cuttings:						
Apical leaves.................	28.39	28.64	14.56	12.52	4.74	4.27
Middle leaves................	27.28	29.11	15.06	13.79	4.11	4.78
Basal leaves.................	27.48	28.19	16.33	14.45	4.45	4.67
Stems.......................	21.67	17.73	20.19	21.49	7.22	7.90
50 Navel orange leaf cuttings.....	29.12	26.63	14.02	13.08	4.44	4.86

Table I gives the soluble calcium, potassium, and magnesium as a percentage of the water-soluble ash in the dry matter. The greatest decrease in calcium occurred in the stem and in the leaf cuttings of the rooted material. The three leaves of the rooted twig cuttings, on the other hand, showed a slight but consistent increase. It would appear at first that the decrease of calcium in the stem can be accounted for by the increase of calcium in the three leaves, and that perhaps no calcium was needed for root development. However, the rooted leaf cuttings where no stem is present also showed a decrease, hence it seems that calcium is translocated during the rooting process.

A general reduction in the percentage of potassium occurred in all of the rooted material, with the exception of the Navel orange stem,

where there was a slight increase. This would also indicate, therefore, that potassium is used in the rooting process. The differences in the percentages of magnesium in control and rooted plants were not consistent. Analytical results obtained for the rootlets were found to be unreliable, owing to adhering sand particles and to the leaching out of soluble salts by washing.

Culture solution studies with rooted lemon leaf cuttings

Mature Eureka lemon leaf cuttings were rooted in washed sand in the propagation frame. Twenty-five similar leaf cuttings were prepared for analysis. After three months the rooted leaves were carefully lifted out of the sand and 25 of these were likewise prepared for analysis. Twenty-five of the rooted leaf cuttings were placed in each of five culture solutions: Hoagland's solution,[3] to which was added: (A) nothing (control); (B) calcium 159 p.p.m., nitrate 493 p.p.m.; (C) calcium 318 p.p.m., nitrate 986 p.p.m.; (D) calcium 477 p.p.m., nitrate 1479 p.p.m.; (E) calcium 636 p.p.m., nitrate 1972 p.p.m. The plants remained in these solutions for three months and then were taken out and prepared for analysis.

It may be mentioned that the leaf cuttings, after having remained in the culture solutions for this period, were curled and showed the prominent veins characteristic of old lemon leaves in the field prior to abscission. This premature aging of leaf cuttings has not been observed in stem cuttings.

It will be seen from table II that the percentage of soluble calcium in the leaves became greater with increasing concentrations of calcium in the culture solution. During the rooting process the percentage was reduced from 16.91 to 15.52. This decrease in the soluble calcium continued in solutions A, B, and C, notwithstanding the fact that the solutions were well supplied with calcium. The soluble calcium of the leaves which grew in solutions D and E showed values that may be considered comparable with those of the unrooted leaves.

[3] Composition expressed as parts per million:

Na	K	Ca	Mg	Fe	Mn	NO₃	Cl	SO₄	PO₄	Total
7	185	159	54	1	0.1	718	10	216	105	1455.1

To this solution traces of elements not ordinarily used in culture solutions were also added.

There was a slight decrease in soluble potassium during the rooting process. After three months in the culture solutions there was an enormous increase, however, the highest value being found in the leaves which grew in solution A and the lowest in those which grew in solution E. The gradient here is reversed as compared with that for calcium. The values obtained for magnesium do not show any appreciable changes.

From the data presented, it would seem that changes in the solubility of calcium and potassium in citrus leaf or stem cuttings occur

TABLE II

SOLUBLE CALCIUM, POTASSIUM, AND MAGNESIUM AS PERCENTAGES
OF WATER-SOLUBLE ASH IN THE DRY MATTER

EUREKA LEMON LEAF CUTTINGS	CALCIUM	POTASSIUM	MAGNESIUM
Not rooted......................	16.91	25.83	5.18
Rooted (not placed in culture solution)........................	15.52	25.64	6.08
Placed in solutions:			
A............................	12.41	59.45	6.36
B............................	13.01	58.64	5.73
C............................	14.73	55.79	5.65
D............................	17.38	48.17	5.73
E............................	18.34	44.05	6.15

during the rooting process. This, together with the enormous increase in soluble potassium when the rooted leaf cuttings were given nutrients, throws some light on the great changes in solubility that may occur under various conditions of growth. The premature aging of leaf cuttings when in culture solutions may be due to an unbalanced condition, brought about by continuous absorption of soluble materials by the rootlets and the absence of an outlet for vegetative growth.

Summary

1. Stem cuttings provided with leaves and single leaf cuttings of Eureka lemon (a variety of *Citrus limonia* Osbeck) and Navel orange (a variety of *C. sinensis* Osbeck) constituted the material for this study. During the rooting process changes occurred in the percentages of soluble calcium and potassium of the water-soluble ash in the dry matter. In the case of stem cuttings, the greatest reduction in soluble calcium was found to occur in the stem, the leaves actually

showing a slight but consistent increase. In leaf cuttings where no stem was present there was also a loss of soluble calcium. In the case of potassium the main loss was sustained by the leaves of the stem cuttings, and a similar reduction also occurred in the leaf cuttings.

2. When rooted lemon leaf cuttings were grown in culture solutions of different concentrations of calcium, it was found that while the soluble calcium content of the leaves did not change to any great extent, the soluble potassium content increased enormously.

3. The solubility of magnesium in the leaves was not affected, either during the rooting process or during subsequent growth in culture solutions.

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BRIEFER ARTICLES

CELL WALL OF VAUCHERIA GEMINATA

During a recent investigation of the methods of healing and wall formation in certain algae and water molds, it was found necessary to study the composition and structure of the wall of *Vaucheria geminata*. In this connection reference was made to a paper by WURDACK.[1] By means of examination in polarized light, and by checking the color reactions, hydrolysis, and the solubility of the membranes, Miss WURDACK found that: the cell wall of *V. geminata* is composed of two layers . . . . pectose and cellulose, the former being on the outside . . . . before treating with the pectose solvents the inner layer reacts negatively to the hydrocellulose reaction. . . . . After removing the outer layer of pectic compounds, the inner layer of the cell wall reacts readily to the cellulose reagents, giving a positive test to the hydrocellulose reaction. . . . . This outer layer is very difficultly permeable to cellulose reagents, particularly those used in the hydrocellulose reactions.

After cutting a filament of *V. geminata* into small sections, the protoplast flows outward, leaving the wall as a hollow cylinder. Through this cylinder the cellulose reagents (iodine potassium iodide and 70 per cent sulphuric acid) may diffuse, and, one must assume, come in contact with the supposed inner cellulose layer. Although innumerable filaments were cut, freed of their protoplasm, and treated with iodine potassium iodide and sulphuric acid, at no time did the inner layer of the cell wall react positively to the hydrocellulose reaction. This would seem to indicate that the inner layer is an extremely thin one, composed either of pectose substances impermeable to the cellulose reagents used or of cellulose heavily impregnated with pectose, which, however, fails to mask the polarized light reaction of the cellulose present.

A cut end of a filament of *V. geminata*, when treated with iodine potassium iodide and 70 per cent sulphuric acid, shows a slight blue color, indicating that there the reagents are in contact with a true cellulose layer.

These facts suggest that the cell wall of this alga is composed of three layers: an outer layer of pectic compounds, a middle layer of cellulose, and an extremely thin inner one either of pectose substances or of cellulose heavily impregnated with pectose.—LADLEY HUSTED, *Oberlin College, Oberlin, Ohio.*

[1] WURDACK, M. E., Chemical composition of the walls of certain algae. Ohio Jour. Sci. 23:181–191. 1923.

CURRENT LITERATURE

BOOK REVIEWS

The Strasburger text

In the early nineties STRASBURGER planned a comprehensive textbook of botany, and he himself wrote the introduction and morphology, including cytology. He associated with himself FRITZ NOLL, who wrote the physiology; HEINRICH SCHENCK, who wrote the cryptogams; and A. F. W. SCHIMPER, who wrote the phanerogams. SCHIMPER, best known for his extensive travels and his work on plant geography, died, and from the sixth edition up to the present time GEORGE KARSTEN has written the phanerogams. NOLL was the next loss, and from the tenth edition, in 1904, up to the sixteenth, in 1923, LUDWIG JOST wrote the physiology. The most severe loss of all came with the death of STRASBURGER in 1912. From the twelfth edition up to the present time the morphology has been written by HANS FITTING, who succeeded STRASBURGER in the Bonn laboratory. SCHENCK's death in 1927 removed the last of the founders of the famous textbook, and his place has been taken by RICHARD HARDER. JOST resigned after the sixteenth edition (in 1923), and HERMANN SIERP wrote the physiology for the seventeenth edition, which appeared in 1928.

The first English edition, translated by H. C. PORTER, of the University of Pennsylvania, appeared in 1898, followed by editions in 1903, 1908, and 1912, all based upon PORTER's translation; but in the present edition,[1] the sixth, which was revised with the seventeenth German edition by Dr. W. H. LANG of the University of Manchester, the changing authorship of the German editions necessitated such extensive revision that PORTER's name is omitted from the title page, and his connection with the English version is described in the introduction. Like the previous editions, the present one is a free translation into idiomatic English, so that only occasionally some peculiar use of a word or some word arrangement makes us realize that our vernacular was not the original language of the text.

It is gratifying to note the increasing recognition given to American work, both in the text and in the illustrations.

Alternation of generations, the cytological basis for which was so well presented by STRASBURGER, is illustrated with comprehensive diagrams covering

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[1] FITTING, H., SCHENCK, H., JOST, L., KARSTEN, G., Transl. by W. H. LANG. STRASBURGER's Textbook of botany. 1921. 6th English ed. 8vo. pp. xi+799. *figs. 833.* Macmillan & Co. 1930.

the subject from the algae to the orchids; but the terms haploid and diploid are unfortunately regarded as synonyms for gametophyte and sporophyte.

Considering the fact that in Germany and England Latin and Greek are still important features of a university curriculum, and that even students specializing in science are likely to know something of these languages, it seems surprising that *macro* is used throughout instead of the more accurate *mega*. Macro means long while mega means large, and is the equivalent of the Latin *magnus*. Macro was doubtless used at first because it sounds as if it were the opposite of micro. Many American texts used the more accurate terms, megaspore, megasporangium, megasporophyll, etc.

The STRASBURGER text won great popularity from the first, partly because it was so authoritative, and partly because it had a unity which is likely to be lacking in a book with several authors. NOLL, SCHIMPER, SCHENCK, and also KARSTEN, were students and friends of STRASBURGER, closely associated with him in the Bonn laboratory, so that the constant contact and discussion prevented repetitions and overlapping which might otherwise have occurred. As one editor has followed another there has been no change in the general plan of the book; the changes for the most part having been such as the progress of the science has demanded. The illustrations have remained about the same. Scarcely any of the figures of the original work have been removed, but new figures have been added until the latest edition, from which the present translation was made, has 833 excellent illustrations. Many of the colored figures illustrate plants which are used for drugs. The official plants are those of the British Pharmacopoeia, instead of those official in Germany, Switzerland, and Austria.

The literature cited has increased from 20 pages in the fifth English edition to 30 pages in the sixth. A study of this list is interesting. In the morphological section, somewhat more recognition is given to American work than in previous editions; in physiology, American work is scarcely recognized at all; but in the treatment of bryophytes and pteridophytes, works in English receive more attention. On the other hand, KARSTEN's list is broadly international in scope, and his treatment of the subject shows the same breadth and grasp of botanical problems. In this edition JOST has been succeeded by SIERP, and SCHENCK's section has been written by RICHARD HARDER. While these two newcomers have made some changes, these do not change the general treatment of the subject. It is to the credit of the various authors of succeeding editions that they have preserved the original plan and unity of the book by presenting the fundamentals of botany, which do not change much with fluctuating theories.

Even where American texts are used in college classes, the STRASBURGER text should be of interest, because it puts the student into contact with German ideas and ideals of botanical science, and a comparison of the numerous editions shows the evolution of botany during the past 25 years, at least as far as it is taught in Germany.—C. J. CHAMBERLAIN.

Methods in plant ecology

A recent number of ABDERHALDEN'S[2] great series on methods of investigation in science is devoted to phases of ecology. In the first portion BEGER[3] discusses the methods in use by the Zurich-Montpellier school of ecology for the analysis of the structure of the plant association. In addition to the descriptions of the methods employed, numerous examples of the application of the methods are given.

The larger portion of the number is devoted to methods employed in the study of plant succession. In it LÜDI[4] sketches the history of the development of the modern concept of plant succession, giving proper emphasis to the part played by the American school of ecology, represented by COWLES, CLEMENTS, and their followers. Such quantitative methods as quadrat studies are described and illustrated by examples from both America and Europe. The advantages of permanent and other quadrats are discussed and exemplified. The methods by which postglacial vegetation has been investigated by means of the fossil pollen from peat bogs are fully described and illustrated by the reproduction of many charts, graphs, and pollen diagrams.

The two papers combine to produce a volume that will be indispensable to all workers in plant ecology.—G. D. FULLER.

Life history of flowering plants of central Europe

A useful and rather detailed handbook,[5] giving the ecological life history of the flowering plants of Germany, Austria, and Switzerland, is in process of publication. To date 36 serial numbers (Lieferungen) have appeared, and a number of additional sections are to be published. The earlier numbers cover the gymnosperms, monocotyledons, and several families of the dicotyledons. The numbers here reviewed include the Salicaceae, Myricaceae, Oxalidaceae, Euphorbiaceae, Tiliaceae, and Orchidaceae. Totally or in part the discussion covers the individual species, while the families and genera in general receive only a short treatment, including a bibliography. The most interesting facts about the individual species are given from a biological and morphological point of view, and anatomical details, as species characters, are added when they are of special

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[2] ABDERHALDEN, EMIL, Handbuch der biologischen Arbeitsmethoden. XI: Chemische, physikalische und physikalisch-chemische Methoden zur Untersuchung des Bodens und der Pflanze. Teil 5, 3:481–728. Urban & Schwarzenberg. 1930. RM 13.50.

[3] BEGER, HERBERT, Praktische Richtlinien der strukturellen Assoziationsforschung im Sinne der von der Zürich-Montpellier Schule geübten Methode. In ABDERHALDEN'S Handbuch der biologischen Arbeitsmethoden. XI; 5:481–526. 1930.

[4] LÜDI, WERNER, Die Methoden der Sukzessionsforschung in der Pflanzensoziologie. In ABDERHALDEN'S Handbuch der biologischen Arbeitsmethoden. XI, 5:527–728. 1930.

[5] Lebensgeschichte der Blütenpflanzen Mitteleuropas. Edited by WANGERIN, W., and SCHRÖTER, C., Lieferung 31–36. Eugen Ulmer, Stuttgart. 1930.

importance. There are ample illustrations, and the handbook should prove of considerable taxonomic value for a student of the central European flora.—A. C. Noé.

A chemistry of protoplasm

A new volume has appeared in the series *Protoplasma Monographien*,[6] dealing with the chemical conditions of protoplasm. In this book KIESEL makes a valuable contribution to biochemistry. In the introductory chapter protoplasm in general is discussed; the following chapters consider cytoplasm and its chemical constituents, the nucleus and its constituents, the nucleus during rest and division, the chemical substances found in the nucleus, protoplasm without nucleus, and finally the plasmodia of *Myxomycetes*. A bibliography of 568 titles is included.—A. C. Noé.

NOTES FOR STUDENTS

Chlorophyll content and photosynthesis.—There are usually distinguished two internal factors affecting the process of photosynthesis: chlorophyll and the enzymatic or protoplasmatic factors. It must be admitted, however, that there are few definite data as to the relation of these factors to this process. Since the work of WILLSTÄTTER and STOLL, we have had reliable methods of determining the chlorophyll content of plant tissues, but the main difficulty of studying the relation of this factor to photosynthesis has been the impossibility of varying experimentally the amount of chlorophyll present. The usual method has been to use green and yellow varieties of the same species of plant, but the investigators could not be sure that the varieties were the same in all respects except their chlorophyll content. We are indebted for most of our knowledge of these two internal factors to WILLSTÄTTER and STOLL. In the case of chlorophyll, although some of their experiments showed a rough correlation between chlorophyll content and photosynthesis, in general there was no definite correlation. They did much work on the effects of variations in light intensity and temperature on the rate of photosynthesis of green and yellow varieties. They found that in the case of green varieties an increase in temperature caused an increase in photosynthesis, while an increase in light intensity had relatively little effect. The yellow varieties, however, were more affected by light than by temperature. They also found that it took a much higher light intensity to get maximum photosynthesis in the yellow varieties than in the green. The different effects of light and temperature on the green and yellow varieties were regarded by these workers as supporting the viewpoint that there are involved in photosynthesis two stages or series of stages, one photochemical and the other chemical.

EMERSON[7] seems largely to have solved the difficulties in studying the effects of variation in the chlorophyll content on photosynthesis, at least in the case

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[6] KIESEL, A., Chemie des Protoplasmas. pp. viii+302. Gebrüder Borntraeger, Berlin. 1930. M. 20.

[7] EMERSON, ROBERT, The relation between the maximum rate of photosynthesis and concentration of chlorophyll. Jour. Gen. Phys. **12**: 609–622. 1929.

of one of the lower plants. He used the alga *Chlorella vulgaris*, and varied the chlorophyll content by growing it in a nutrient solution of varying iron content, and, in order to get healthy cells of normal metabolism in the case of cultures of low iron content, he used a nutrient solution containing about 1.5 per cent glucose. With glucose in the culture solution, it was found that by varying the iron content, cells of nearly the same size and same rate of respiration, but of very different chlorophyll content, could be obtained. With material secured in this way EMERSON found that as the chlorophyll content increased photosynthesis increased proportionally. The curves obtained by plotting the rate of photosynthesis against chlorophyll content were practically straight lines.

By other experiments, EMERSON[8] found that contrary to the results of WILLSTÄTTER and STOLL, the maximum rate of photosynthesis was reached at about the same light intensity no matter what the chlorophyll content. The actual rate of photosynthesis was much higher in the case of the higher chlorophyll content. Also, over this same range of chlorophyll content the process of photosynthesis showed about the same relationship to temperature. The chemical phase of photosynthesis, the part that has been thought of as enzymatic in nature, has been regarded as being readily inhibited by prussic acid. EMERSON, however, found that prussic acid inhibits photosynthesis more in the lower chlorophyll concentration than in the higher. The results of this paper are really not in accordance with the viewpoint that photosynthesis is made up of photochemical and chemical phases. EMERSON suggests that perhaps chlorophyll plays a part, not only in the photochemical phase but also in the chemical, and that photosynthesis may involve an autocatalytic reaction.

If these results are substantiated, it would seem that EMERSON has worked out a method which should enable investigators to study more accurately the relation of chlorophyll content to the process of photosynthesis than has been possible in the past.—S. V. EATON.

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[8] EMERSON, ROBERT, Photosynthesis as a function of light intensity and of temperature with different concentrations of chlorophyll. Jour. Gen. Phys. **12**: 623–639. 1929.

ECOLOGY OF THE MOSSES OF GRAND DE TOUR REGION OF ILLINOIS, WITH SPECIAL REFERENCE TO pH RELATIONS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 414

CHARLES E. MONTGOMERY

Introduction

Work on soil reactions with respect to plant distribution has progressed greatly during the last few years. In a short review, WHERRY (31) states that although the first paper on soil reactions and natural plant distribution appeared in 1916, during the following decade more than 500 papers were published on this general subject. Among early workers, HEALD (11) found that the positive ion of weak acids and soil salts produced a toxic effect on seedlings. Following this lead, many theories concerning the causes of soil acidity were rapidly developed. HARRIS (9) reviewed these in 1914, and suggested that soil acidity was due to the fact that colloids adsorbed the negative ions from soil salts, leaving the positive ions to give the acid effect. WHERRY (26) described soil acids as both organic and inorganic, and indicated that colloids were indirectly effective.

The importance of total soil acidity soon gave way to the idea of H-ion concentration. GILLESPIE (5) discussed acid intensity, H-ion concentration, as a more potent factor in plant growth than total acidity. WHERRY (25, 27–30) has demonstrated the effect of the H-ion concentration upon many of the various groups of the higher plants. KURZ (14) showed the H-ion to be an important ecological factor in the different soils of the Chicago region. SALISBURY (19)

found that in old forest soils the top layer contained the higher H-ion concentration, which decreased in the lower layers and finally became alkaline. The alkaline materials of the top layers leach more readily than the acid and are therefore carried to the lower strata, leaving the acids above. Thus soils in different places show the variable reaction which is a basic factor in plant distribution. OLSON (17) found that as a rule the availability of some soil nutrients is not affected by the H-ion, and therefore the H-ion concentration itself is an important factor in plant groupings. Although WHERRY discovered that northern ferns generally prefer alkaline soils and southern ones prefer acid, STEAGALL (22) did not find that the ferns of Illinois showed these preferences. ROSEN (18) found a bacterial disease of foxtail sensitive to the H-ion concentration of the media.

Some experimenters have taken a slightly different angle of the problem. HARTWELL (10) noticed that the sap of flat turnips was more acid when grown in soils of a higher phosphorus content. TRUOG (24) states that alkaline substances of the soil caused plants to have higher alkaline contents. HABER (8), working with the tomato, found that the contents of the plant were affected proportionately to the acid or alkaline substances of the soil. The roots seemed to be most affected and the fruits least.

There is less literature on mosses in relation to the H-ion concentration. SKENE (20) found sphagnum sensitive to acids and bases both in substrata under natural conditions and in artificial cultures. KURZ (15), working on bogs, concluded that sphagnum and associated mosses were the cause of bog acidity. His conclusions were based on the fact that bogs where these mosses had not appeared were not acid but alkaline in reaction. AMANN (1) classified mosses as acid, subacid, neutral, and alkaline. He used the colorimetric method to test the soils, but there is no indication concerning the accuracy of his work. TAYLOR (23) studied the succession of mosses on various habitats in the Chicago region, and found an interesting correlation from this angle. DAVEY DE VIRVILLE (2) studied the relation of mosses to environment. He experimented on such ecological factors as sunlight, water, temperature, humidity, and soils, but did not touch upon the H-ion concentration.

The purpose of the investigation here reported was to discover the

H-ion concentration relationships between the mosses and their substrata in the region of Grand de Tour, Illinois, this vicinity offering a good variety of both mosses and habitats. In the beginning it was thought necessary to make tests only of the top centimeter of soil and the moss parts that lay upon the surface, but later it was found desirable to add readings of the soil 2-3 cm. deep. The first group of tests included such materials as moss bases, rhizoids, humus, and soil particles; the second group usually consisted of soil materials only, exclusive of moss parts and humus. In the original plan the intention was to gather data that would show to what extent if any the mosses affected the H-ion concentration of the soil. From evidence accumulated in this work, and from the work of KURZ (15), it seems likely that there is a contribution to the soil from the moss, but upon further study it was found that data gathered in this way could not be used in the solution of the problem. This part of the work had to be abandoned therefore, since it required a special method and a much longer time than the other parts of the program.

Finally the work resolved itself into the four following phases: (1) to discover the H-ion concentration of the mosses and the top soil to which they were attached; (2) to discover the H-ion concentration of the soil 2-3 cm. deep; (3) to see whether there was any correlation between (1) and (2); (4) to determine the H-ion range of some of the more common mosses of the region. The work on the last part was necessarily limited, because in making the tests the mosses were taken as they naturally occurred in the field, and no effort was made to get a certain large number of tests for any moss or group of mosses.

Investigation

METHODS

With the exception of a few samples brought to the laboratory for checking, all tests were made in the field. The Morgan Soil set (16) was used throughout to test both the soils and moss materials. This is a colorimetric tester developed to an accuracy of 0.2 pH. It consisted of a porcelain block arranged in such a manner that when the soil was placed in part of a groove the indicator could be dropped into another place and allowed to run through it into a small pit. A color chart was then used to read the results. The indicators em-

ployed in this apparatus were phenol red, bromthymol blue, chlorphenol red, and bromcresol green. The complete range of the set was from 8.2 in phenol red to 3.8 in bromcresol green. At first tests were made of the top soil and the moss materials on the surface, but later tests of the soil 0.5-1 inch deep were also taken. If there was any doubt about the results in any case the test was repeated until a constancy was reached. In order to insure greater certainty, tests were made in the field, in the laboratory, and in both wet and dry conditions. Check tests were also made of the same plot at different times throughout the season. Moss samples were collected and brought to the laboratory for identification. The manuals of GROUT (6, 7) and DUNHAM (4) were used in this work.

All readings were taken in pH values because the color charts were marked in those figures. Thus the color chart for bromcresol green read 3.8-4.0-4.2-4.4-4.6-4.8-5.0-5.2-5.4-5.6, and the others followed the same scheme with a slight overlap on the indicator to each side. The pH values translated to units of H-ion concentration are as follows: the pH value of 3 equals the H-ion concentration of 0.001 (gm. H per liter), 4 is equal to 0.0001, 5 to 0.00001, 6 to 0.000001, 7 to 0.0000001 (the neutral point), and 8 to 0.00000001 (an alkaline reaction). Altogether there were a few over 1100 recorded tests. These are charted and listed in such a manner as to show the pH range of each area studied and the pH range of the different species found. In connection with the latter point, there were many species for which so few readings were taken that these can be considered only suggestive and not in any way conclusive.

GRAND DE TOUR REGION

The field covered in this survey included the Rock River bluffs and valleys in the vicinity of Grand de Tour, Illinois, some bluffs near Castle Rock and the Devil's Backbone, and the Illinois State Park known as The Pines. These places are all in the region called the Dixon quadrangle, as described by KNAPPEN (13) of the Illinois State Geological Survey. The region offered a wide variety of geological and ecological situations, such as gentle slopes, steep hillsides, valleys, open spaces, wooded spots, flat hill tops, and faces of hillsides in almost every direction. From a geological point of view the

region has exposures of St. Peter sandstone, Platteville limestone, and the Illinois glacial moraine. These materials are in various stages of erosion and are more or less mixed with humus from the plant cover.

St. Peter sandstone and the eroded sand from it form the basic soil for all the territory near Grand de Tour, Castle Rock, and the Devil's Backbone. This is found in open exposures and with different types of cover. It is in the realm of the oak-hickory climax and these woods occupy practically all the places studied. The north slopes and other protected places are usually coated with a layer of humus of varying degrees of thickness. These are in various stages of meso-phytism and are covered with a wide variety of higher plants as well as mosses. Rock and sand outcrops are frequent, both on the tops of bluffs and hillsides. Such places are xerophytic and support a sparse vegetation.

The Pine Woods is a remnant of native white pine timber that has not only been able to maintain itself against the competition of other species, but is actually reseeding the parts that have been occupied by the oaks. The trees are rooted in a shallow layer of Illinois glacial till of varying depths. This overlies Platteville limestone which out-crops in the bluffs along Pine Creek. The creek has cut a deep valley through the park, forming steep bluffs that give a variety of places for the growth of plants. The pines begin at the top edge of the east bluff and extend eastward in varying widths throughout the length of the park. Oak-hickory woods occupy a large portion of the rest of the region. The east bluff is very mesophytic, and is covered with a thick growth of vegetation except in small sections of a vertical cliff. The west bluff is drier and has much more rock exposure. Cedars are prominent trees along this bluff.

This region lies in the cool temperate belt and has an average annual rainfall of 30–35 inches. The annual mean temperature is around 45° F. Climatic changes are often sudden and at times severe, but not so hard that the ordinary vegetation for this region ever fails to develop. DeForest (3) presented data on precipitation and temperature over a period of 62 years. He thought that during that period there was a slight drop in precipitation, but otherwise no noticeable change in climatic conditions.

Babson Hollow

In the woods along the river northwest from Grand de Tour, a small stream has cut a deep valley in the ridge parallel to the river edge. It runs in a westerly direction for a short distance, forming a typical mesophytic north-facing bluff about 50 feet high. This bluff is covered with oaks, walnuts, elms, and cherries. In the spring hepaticas, bloodroots, trilliums, claytonias, and many other common wood plants are abundant. Throughout the growing season parts of this slope are well matted with luxuriant growths of mesophytic mosses. Many of them scarcely check their growth through the drier months of summer, because of the very regular supply of water. Evaporation rates are always low because sun and wind have no chance to reach this secluded spot. In many places the mosses are in a pure stand while in others they represent a wide variety of mixtures.

At the top of the bluff, in an open place covering several square yards, are a few patches of *Polytrichum commune* and *Catharinea angustata*. These mosses are growing luxuriantly and are found in practically pure stands. Small bits of forest débris have settled among them, but not enough to interfere with their normal growth habits. This is the driest part of the whole plot, owing to the fact that the moisture supply is not so good as farther down the hillside and evaporation is more rapid. On this account growth ceases during the dry parts of the season.

Farther down the hillside different groups of mosses appear. Those that occur most commonly are *Catharinea undulata, Bartramia pomiformis*, and *Aulacomnium heterostichum*. In places they extend well to the bottom of the slope. *Bartramia* is especially abundant in the steeper shaded areas in both pure stands and mixtures. These mosses are rather hardy, and are prominent throughout the growing season. Other mosses that grow luxuriantly at different parts of the season are *Mnium cuspidatum, M. hornum, Hylocomium triquetrum, Thuidium recognitum, Eurhynchium serrulatum, Brachythecium oxycladon*, and *B. rutabulum. Eurhynchium* and *Brachythecium* occur all the way to the bed of the stream and may be found on soil and dead wood alike.

The underlying soil of this slope is St. Peter sandstone. In places it is covered with a layer of humus on which the mosses grow; in

others the moss mat is directly upon the sand and forms its own layer of humus. The source of water is not only from precipitation but from seepage and capillary movement. These give an ample supply which is well conserved for the heavy plant cover.

The H-ion concentration on this slope was characteristic of hill-sides in general. Near the top, in the more or less level beds of *Polytrichum* and *Catharinea angustata*, the moss tests ran between 5.0 and 6.0 and the soil 1 inch deep held between 4.0 and 5.0. Farther down the slope, in mixed beds of *Aulacomnium, Hylocomium, Bartramia*, and *Mnium cuspidatum*, the moss tests rose to a range from 6.4 to 7.6, while soil tests rose to 5.2. At the foot of the bluff and in the stream bed the moss tests reached 8.0 and 8.2, but the soil gave tests from 6.0 to 8.0. The entire range for the mosses on this slope

TABLE I

BABSON HOLLOW; pH TESTS OF MOSSES AND TOP SOIL

Species	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0	8.2
Polytrichum commune		3	1	3	3	2	1	1	3	3	1	2		1						
Catharinea angustata		1		2		2	1			1	1									
Catharinea undulata							1		3	1	1	1		9	2	5				1
Bartramia pomiformis						1	1				1	2	3	7	2	3	1			1
Aulacomnium heterostichum									1	3				3	5	3				1
Mnium cuspidatum				1	1					1	1	1	1	9	5	3				
Mnium stellare					2	1					1	1			1					1
Thuidium recognitum														2	1	1				
Brachythecium oxycladon											1			3						1
Brachythecium rutabulum								1							1					
Amblystegium serpens																	1			2
Eurhynchium hians															1					
Eurhynchium serrulatum														2		2	1	1		4
Pylaisia schimperi					2															

was from 5.0 to 8.2; for the soils it was from 4.0 to 8.0 (table I). The acid mosses were confined to the top of the hillside on the higher acid soils. The alkaline groups were found from the middle to the base, and were distributed on both acid and alkaline soils. The pH correlation between the soil and mosses on this slope was close. The readings of the mosses generally varied 0.0–2.0 points above those of the soils on which they grew.

BABSON BLUFF

Rock River has cut a steep bluff for several rods along the south edge of the Babson wood. This bluff is an outcrop of St. Peter sand-

stone which is badly eroded into pockets, shelves, and crevices of
many descriptions. In most places loose sand covers the rock, but
at times the crumbly rock is bare. The top edge of this bluff is semi-
xerophytic and for this reason was included in this study. Thin lay-
ers of humus have accumulated in pockets and other protected
spots. Disintegration and erosion are rapidly changing the surface
layers in many places. These features, together with the dry condi-
tions, make an unfavorable situation for moss growth.

Vegetation along the edge of this bluff is not dense. Oaks and
hickories are the dominant trees, with a fringe of junipers occupying
the more exposed and difficult sites. Few of the herbaceous forms
find a place to lodge. *Draba* sp. may be found along the shelves and
paths, and *Viola pedata* is widely scattered over the slope above. On
the more level spots *Amphicarpa monoica* attains a moderate
growth in the late summer. There are no dense coverings of any kind
and all plants reach only an average size. Mosses find this a difficult
place in which to get a foothold. Some have been able to lodge on
the narrow rock and sand ledges, and others have become well estab-
lished on the stable surfaces under trees and in the more protected
pockets. Although *Polytrichum commune* and *Catharinea angustata*
have been able to spread over rather large patches, other mosses are
found only in small clumps. None of the species have made the
growth here that they have in more mesophytic places. Under the
cedars and in more protected spots were well developed mats of
Brachythecium oxycladon. This moss has a liking for neutral to alka-
line soils, and on the sandy soil of this bluff maintains a range from
pH 7.0 to 8.2. Sand shaken out of the rhizoids tests 7.0 and 7.2, but
1-inch deep tests range between 5.0 and 6.0. Other mosses occurring
on this bank are *Catharinea undulata, Pohlia nutans, Mnium spinu-
losum, Bryum binum, B. argenteum, B. caespiticium, Encalypta
ciliata*, and *Ceratodon purpureus.*

The pH readings of this region showed a wide variation and an
unusual irregularity. *Polytrichum commune* ranged from 5.0 to 7.4,
and *Catharinea angustata* varied from 6.0 to 8.0. Vertical slopes
covered with *Mnium spinolosum, Encalypta ciliata*, or *Brachythecium
oxycladon* were always neutral or alkaline, but if *Pohlia nutans* or
Dicranum scoparium formed the moss cover the tests were regularly

acid. The entire range of this bluff for the mosses and top soils was 4.6–8.2; for the soils 1 inch deep it was 4.2–7.2. A complete list of the mosses found in this area with the range of their tests is given in table II.

TABLE II

SPECIES	RANGE OF pH	
	Moss and top soil test	Test of soil 1 inch deep
Brachythecium oxycladon.........	6.0–8.2	5.0–7.4
Bryum argenteum................	7.6–8.2	8.0
Bryum roseum...................	7.6	7.4
Bryum caespiticium..............	5.4–7.6	6.2
Bryum binum....................	8.0	....
Mnium spinulosum...............	6.4–7.2	....
Catharinea angustata............	6.0–8.0	5.6–7.4
Polytrichum commune............	5.2–7.4	5.6–6.2
Barbula unguiculata.............	7.4	....
Tortula ruralis.................	7.8	....
Ceratodon purpureus............	4.6–7.6	5.2–5.6
Encalypta ciliata...............	7.6	....
Pohlia nutans..................	5.2	....
Dicranum scoparium.............	4.4–6.0	4.4
Ditrichum pallidum.............	7.4	6.2
Funaria hygrometrica...........	7.4	6.2
Thelia lescurii.................	7.0–7.2	7.2

BLACK HAWK BLUFFS

South and west of the county line bridge below Grand de Tour are three large moss plots that present very different situations. One is a dry hilltop with a mesophytic slope, another is a mesophytic shelf on the north side of a steep bluff, and the third is a wet hillside.

A few rods west of the bridge close by the river is a prominent outcrop of sandstone. The top is dry and open, with only a thin layer of sand covering a part of the rock. On the eastern edge are a few dwarf oaks, and on the north and west edges are some straggling specimens of *Pinus strobus* and *Betula lutea*. Huckleberries are able to find rooting in the large cracks of the rock, and *Draba* sp. and *Viola pedata* are the most common species of herbaceous plants. The south slope is gradual and not difficult of approach. Characteristic plants are *Ranunculus fascicularis*, *Antennaria alternifolia*, *Draba* sp., *Viola pedata*, *Synthyris bullii*, and a few hardy grasses. The east slope from the base almost to the top is mesophytic. *Quercus rubra*

and *Hamamelis virginiana* form the tree covering, and vigorous plants of *Arisaema triphyllum, Smilacina* sp., *Hepatica triloba,* and *Aralia nudicaulis* are the most common smaller forms.

At the foot of the east side the most widely distributed moss was *Mnium cuspidatum.* Other mosses that occurred in scattered patches were *Bryum caespiticium* and *Brachythecium oxycladon.* These mosses ranged from 7.4 to 8.2, and grew on a substratum that tested from 6.8 to 7.0. Near the middle of the hill were large mats of *Thuidium recognitum, Catharinea undulata,* and *Polytrichum commune.* These grew on thin layers of sand and over the edges of rock shelves. They ranged from 5.4 to 8.2. On the ledges and the summit of the bluff the species that occurred most frequently were *Polytrichum commune, P. piliferum, Catharinea angustata, Pohlia nutans,* and *Dicranella heteromalla.* The range was 4.2–7.8 while the substratum tested 4.4–7.2. Some noticeable irregularities occurred on this bluff. *P. piliferum,* which usually grew on highly acid soils, tested 7.2 on sandy soil with a range of 5.0–7.2. *C. angustata* tested 7.4 on soil of 7.0; *P. commune* and its soil both ran to 7.2. On the south slope were some large plots of *P. commune* mixed with a few specimens of *Thuidium recognitum, Bartramia pomiformis,* and *Brachythecium oxycladon.* These all tested from 7.2 to 7.8 and the soil ran 4.4–7.2. The tests of the soil usually ran about 7.2, but one spot was found in which the tests were 4.4.

These irregularities are probably caused by different water movements through the rocks and soil. In some places the water comes out as seepage from the layers above, giving a soil with an alkaline reaction. In others the water runs down through the soil, leaving acid conditions at the surface. A few rods south of this bluff is a steep hillside about 45 feet high covered with a thick carpet of mosses. Through the season water seeps down this hill, giving a good supply of moisture to the moss cover. Close around the moss bed are such plants as *Thalictrum dioicum, Smilacina* sp., *Hepatica triloba, Geranium maculatum,* and *Sanguinaria canadensis.* Trees grow near, so that direct sunlight does not fall upon the moss bank for any length of time during the day.

The base of the slope is well covered with *Catharinea undulata* intermixed with *Conocephalus* and *Bartramia pomiformis.* From 6 to 10 feet up *Catharinea* gives way to *Bartramia* and *Thuidium recog-*

nitum. Higher up *Mnium cuspidatum*, small clumps of *Polytrichum commune*, *Aulacomnium heterostichum*, *M. hornum* and *Anomodon attenuatus* complete the cover. These belts are adjusted rather closely to the moisture supply of the hill, with the drier part at the top.

Most of the pH tests of the mosses on this bank were alkaline. In one small plot of *Bartramia*, *Thuidium*, and *Polytrichum commune* the tests were 5.8 to 6.6, but all the others ranged from 7.0 to 7.8. Soil tests ranged from 6.0 to 7.0. The soil below these mosses was almost pure sand with a thin layer of humus formed by the mosses themselves.

A few rods farther south of this same bluff is another moss bank of a different type. It is a broad talus shelf with vertical cliffs above and below. The soil is pure sand held in place by moss beds and humus from the trees. It is a north slope completely shaded by oaks and the cliff above. The north edge is bordered by a number of scrubby pines.

The most interesting moss on this shelf was a luxuriant bed of *Climacium americanum*. A large patch and several small ones had become established near the base of the shelf. *Polytrichum commune* also grew well in the surrounding spaces. Other mosses found on the slope and cliff above were *Catharinea angustata*, *C. undulata*, *Pohlia nutans*, *Mnium cuspidatum*, *Bartramia pomiformis*, *Aulacomnium heterostichum*, *Thuidium recognitum*, and *Brachythecium oxycladon*. These showed their usual reactions when tested. *C. angustata* ran 5.2 on soil with the same test; *P. nutans* tested 4.6 on a sand wall of 4.2; *P. commune* ranged from 6.4 to 7.0 and the soil 1 inch deep tested from 5.4 to 7.0; *Climacium americanum* tested from 6.6 to 7.6, and the soil below ran from 5.2 to 7.0. Other mosses ranged 7.2–7.6 with soil tests varying from 4.8 to 6.8. Although the moss tests were rather regular the soil seemed to be more widely variant. The correlation between the mosses and soils was close in some cases but in others there was a wide difference (table III).

TREE MOSSES

Tree mosses in the Rock River region were not abundant nor vigorous. Three widely separated groups of trees were found to have somewhat representative species, and a brief survey of these was taken.

One wood, an old floodplain along the Black Hawk bluff, is a region of the mesophytic type. The common trees are *Ulmus americana, Celtis occidentalis,* and *Juglans nigra.* These are scattered so that there is plenty of sunlight for the grass cover underneath. The soil is chiefly a sand till washed in from the bluff above, mingled with silt and humus. A few partially decayed stumps and old logs made good substrata for mosses. On tree bases, old wood, and soil surrounding them are such species as *Anomodon minor, Amblystegiella adnata, Brachythecium oxycladon, B. rutabulum,* and *Mnium*

TABLE III

SPECIES	RANGE OF pH	
	Moss tests	Test of soil 1 inch deep
Polytrichum commune............	5.0–7.4	4.4–7.4
Polytrichum piliferum............	5.6–7.4	5.0–6.2
Catharinea angustata............	5.4–7.4	5.4–6.0
Catharinea undulata............	6.0–7.6	5.4–6.8
Ceratodon purpureus............	7.4	6.4
Dicranella heteromalla............	4.8	....
Pohlia nutans..................	4.2–4.6	4.2
Aulacomnium heterostichum.......	7.4–7.8	5.2–5.4
Bartramia pomiformis............	7.2–7.8	5.4–6.0
Bryum argenteum................	8.2	....
Mnium cuspidatum..............	7.6	5.4
Mnium hornum.................	7.4	....
Thuidium recognitum............	5.8–7.4	4.8–7.4
Anomodon attenuatus............	7.0–7.8	7.0–7.2
Brachythecium oxycladon..........	7.4–7.6	5.2–7.2
Climacium americanum............	6.4–7.8	5.2–7.2
Entodon cladorrhizans............	7.4	8.2
Pylaisia schimperi...............	7.4	....

cuspidatum. The pH tests for mosses and their substrata were all alkaline, ranging 7.0–7.8. This is the usual condition for areas of this kind.

The uplands behind the floodplain have been cleared of underbrush and are occupied by pasture grasses. Oaks and hickories are left standing but do not interfere with growth of the grasses. The soil is sand mixed with a small amount of silt and humus. Mosses grow generally on the soil but some are found on the bases of the trees. Those occurring most frequently are *Anomodon attenuatus, Brachythecium oxycladon, Ceratodon purpureus,* and *Polytrichum commune.* The pH readings of the mosses ran 7.4 and 7.6 on soil that tested from 5.4 to 6.8.

About one mile to the southeast of the county-line bridge is a small open group of trees (oaks and hickories) supporting a few tree mosses. The soil is a gravelly till from the Illinoian ground moraine, sloping gently to the northwest and covered with a thin layer of leaf humus. Mosses find a footing on the soil, tree bases, old logs, and stumps. The most abundant species are *Anomodon attenuatus*, *Amblystegium serpens*, *A. kochii*, *A. adnatum*, *Entodon cladorrhizans*, and *Mnium cuspidatum*. The tests for the mosses, bark, and soil ranged 7.2–7.6 except in one case where the moss and bark went to 6.8.

The third wood studied for tree mosses was a portion of the eastern part of the pine-oak mixture at The Pines. Part of the work was in a thick growth of oaks with a heavy undercover, part in open timber, and the rest in a thick group of young pines. The soil was sand and gravelly till of the Illinoian ground moraine, covered generally with a layer of humus. In the oaks *Psedera quinquefolia*, *Celastrus scandens*, *Galium* sp., and many other forms hid the ground except in spots. Under the pines needles made such a thick coating of humus that few other plants could get a start. On tree bases, old logs, and stumps among the oaks were found *Mnium cuspidatum* and *Amblystegium serpens*. These tested from 7.0 to 8.2 on soils testing from 4.8 to 7.0. In the young pines the mosses were confined to the bases of trees and barren soils. The prevailing species were *Brachythecium rutabulum*, *Amblystegium serpens*, *Amblystegiella adnatum*, *Bryum roseum*, and *Catharinea undulata*. Tree mosses tested from 6.8 to 7.6 and soil mosses ran between 5.4 and 6.4. The pH of the soil under these pines varied from 4.0 to 7.0. A few small clumps of *Dicranella heteromalla* at the base of the larger pines ran to 4.4 on soil with a pH of 4.2. In all cases where a moss was found both on tree base and soil the soil specimen tested more acid. One case of *A. adnatum* on the bark of a pine showed a reading of 5.4, which is unusually low for tree mosses. In general these tests showed that tree mosses of this region were from neutral to the limit of the alkaline scale. Individual tests in other plots corroborated these data (table IV).

PINE WOODS

About 6 miles east of Polo, where the Burlington Railroad crosses Pine Creek, is the last important vestige of native white pines of north-

ern Illinois. At this point the creek has cut a valley 50–100 feet deep with vertical cliffs and steep slopes on the sides. The pines occupy a narrow strip along the east bluff for a distance of about 1 mile. Not only have the old pines been able to maintain themselves, but young trees are springing up among the oaks farther back, making good headway toward resetting other parts of the woods in pines. Mosses do not form a noticeable part of the pine wood flora; therefore the major effort of this study was spent along the bluffs of the valley.

TABLE IV

	RANGE OF pH	
SPECIES	Moss test	Test of soil 1 inch deep
Catharinea crispa.................	7.6	7.6
Catharinea angustata.............	5.4	....
Catharinea undulata.............	5.6–6.4	....
Dicranella heteromalla.............	4.2–7.0	4.4–6.8
Physcomitrium turbinatum........	6.0	....
Bryum roseum....................	6.2–7.6	....
Mnium cuspidatum...............	7.4–8.2	7.4–8.2
Anomodon minor.................	7.6	....
Anomodon rostratus..............	7.4–7.6	....
Anomodon attenuatus.............	7.4–7.6	....
Brachythecium oxycladon.........	7.6	....
Brachythecium rutabulum.........	5.4–6.8	4.2–6.0
Amblystegium adnatum...........	7.2	7.2
Amblystegium serpens............	7.2–8.2	5.2–8.2
Amblystegiella adnata............	7.2	....
Entodon cladorrhizans............	7.4	....
Entodon seductrix................	7.0–7.2	....

The bluffs are steep slopes covered with enough soil to support a good growth of oaks, elms, hop hornbeams, and cedars, with pines on the upper edge or they are vertical cliffs of solid limestone (Platteville) which carries a scant mixture of lichens, cliff brakes, mosses, and liverworts. The soil of this region is till from the Illinoian ground moraine. Along these bluffs it is a thin layer, but in many places it is covered with a mesophytic vegetation of *Claytonia virginica, Ranunculus septentrionalis, Arisaema triphyllum, Dentaria* sp., *Viola* sp., *Podophyllum peltatum,* and others of this type.

WEST BLUFF

The west bluff comprises a large area, including a part of the bluff of the main stream, cliffs, banks, and other plots along a small tribu-

tary stream that cuts through it from the northwest. The mosses of this area are practically all soil and rock species. On the whole the habitat is dry and thinly covered with vegetation.

The portion of the bluff along the main stream is a precipitous slope with a south face. The soil is thin except at the base, where it supports a growth of walnuts and elms. At the top limestone outcrops are numerous, and the most common tree found here is the cedar. Under the cedars a few poor specimens of *Aquilegia canadensis* have been able to get a footing on the ledges. Lower, *Aquilegia*, *Thalictrum dioicum*, *Rubus occidentalis*, and *Viola cucullata* are representative species. The slope is well shaded and the sunlight reaches only small spots for short periods each day. During the wet seasons mosses grow vigorously but in the summer they are usually dormant. The common mosses are *Grimmia apocarpa*, *Amblystegium serpens*, and *Mnium cuspidatum*. The substratum is soil, rock, shallow soil, and decaying wood. They form thin mats only and do not add much to the humus layer. Because these mosses are growing so close to the limestone surface, all pH tests were alkaline, ranging 7.6–8.2. The correlation was very close, since the soil layer is usually the top material to which the mosses were attached.

Several rods west of this bluff a small tributary flows in from the northwest. It has cut a deep valley in the limestone, leaving rocky cliffs and soil banks on which many mosses have become established. On the rocky cliffs the cedars are the only trees that can grow, but in the valley and on the lower slopes walnuts, elms, hackberries, and oaks are common. Grasses, *Aquilegia*, *Galium* sp., *Ranunculus fascicularis*, *Cynoglossum officinale*, *Viola cucullata*, and dwarfed strawberries make up the ground flora. The substrata are limestone and disintegrated rock with alluvium in the valley.

The mosses growing on the ledges and surfaces of the rock exposures were *Brachythecium oxycladon*, *B. rutabulum*, *Anomodon rostratus*, *Grimmia apocarpa*, *Barbula unguiculata*, and *Mnium cuspidatum*. On account of the dry and hard conditions all mosses showed thin mats of scanty growth. The pH readings were all highly alkaline, 8.0 and 8.2, owing to the presence of limestone. In the valley *Bryum caespiticium* was the only moss that grew in abundance. It tested 8.0 as well as the soil. The soil mosses of the bluffs were *Bryum caespiticium*, *Brachythecium oxycladon*, *B. rutabulum*, *Catha-*

rinea undulata, and *Ceratodon purpureus.* Some of these grew in dense mats in the open spaces between the trees. These mosses ranged between 6.8 and 8.2 with the median on the alkaline side (table V). One group of mosses consisting of *Thuidium recognitum, Polytrichum juniperinum,* and *Catharinea angustata,* growing on a gravelly moraine tested from 5.2 to 5.8. The soil just below the mosses ran from 5.2 to 5.6. These mosses were well established and grew rapidly during the wet seasons. In this territory the mosses were closely confined to soils of their liking: the acid mosses held to acid soils and the alkaline species were found in their respective conditions.

TABLE V

WEST BLUFF AT PINE WOODS; TESTS OF MOSSES AND TOP SOIL

SPECIES	RANGE OF pH																
	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0	8.2
Brachythecium oxycladon																	5
Brachythecium rutabulum												1					
Anomodon minor														1			
Anomodon rostratus																	1
Amblystegium serpens																	1
Grimmia apocarpa														1			2
Barbula unguiculata																	1
Bryum caespiticium															1		
Mnium cuspidatum														2	1		
Polytrichum juniperinum				1	1												
Catharinea angustata		1		1					2				1				
Ceratodon purpureus			1														
Thuidium recognitum				2	1												

EAST BLUFF

The east bluff has a long steep slope covered with a layer of glacial till and disintegrated rock. It has little rock exposure except in one small section where the limestone forms a vertical cliff. The whole bluff is mesophytic in character, even the rock cliff being moist enough in parts to support a growth of mosses and liverworts. The trees of this hillside consist of *Ulmus americana, Quercus alba, Q. rubra,* and *Ostrya virginiana,* with black walnut and red cedar at the base and the pines at the top. Since the ground vegetation is thick, the mosses find it difficult to become established. Small open

spaces, loose rocks, old logs, and tree bases are the spots on which they have been able to grow. The shade is dense and moss mats are usually small, thin, and not very vigorous.

The mosses growing in the edge of the pines at the top of the slope showed a different reaction from those farther down. They consisted of *Dicranella heteromalla* on soil at the base of pines, *Catharinea undulata*, *Ditrichum pallidum*, and a few specimens of *Mnium cuspidatum* and *Brachythecium rutabulum*. *Dicranella* was on soil that tested from 3.8 to 5.3, *Catharinea* ran between 6.2 and 7.4 on soil testing 5.3–6.0, and the other mosses ranged 6.0–6.8 while the soil varied around 5.8 and 6.0.

The reactions from a few yards below the top to the base were all from neutral to 8.0 in the alkaline scale. The mosses were chiefly pleurocarps consisting of *Brachythecium oxycladon*, *B. rutabulum*, *Amblystegium serpens*, *A. varium*, *Anomodon attenuatus*, *A. apiculatus*, *Pylaisia schimperi*, *Entodon cladorrhizans*, *Fissidens taxifolius*, and *Mnium cuspidatum*. *B. oxycladon*, *Mnium*, *Pylaisia*, *A. varium*, and *Entodon*, all growing on rotting wood and soil nearby, tested 5.6, 5.6, 5.2, and 6.8 respectively. All mosses found on soil or rock, including some of these, tested from 7.0 to 8.0. The soil tests were all within the upper one-half inch, and were included with the mosses. The acid soils at the top were characteristic of pinewoods, and the alkaline reactions on the lower slope were the usual type for a region of this kind. The most striking feature about this habitat so far as mosses are concerned was that the pleurocarp forms seemed to be better adapted to a shady, mesophytic, alkaline hillside such as this than the acrocarp forms.

A small section of the east bluff breaks into a vertical limestone cliff about 40 feet high. Three or four feet of glacial till cover the top edge, giving rooting for the large pines that grow close to the margin. Along a portion of the cliff there seems to be a seeping of water which runs down over the side, giving moisture to a thick coating of plants. These consist of *Conocephalus* (*Conocephalum conicum*), mosses, and such flowering plants as *Asarum canadense*, *Arisaema triphyllum*, *Hepatica triloba*, *Dicentra* sp., and *Mitella diphylla*.

The common mosses on the cliff and the talus at the foot are *Brachythecium oxycladon*, *Anomodon attenuatus*, *Mnium cuspidatum*,

and *Bryum roseum*. In places the mosses make a thick mat that covers the rock completely. The pH tests are all alkaline, 7.6 to 8.2 (table VI).

TABLE VI

SPECIES	RANGE OF pH	
	Moss tests	Test of soil 1 inch deep
Catharinea undulata.............	5.2–7.4	6.0–7.4
Dicranella heteromalla...........	3.8–4.6	3.8–4.6
Ditrichum pallidum..............	6.2–6.4	....
Pottia truncata..................	6.4	....
Physcomitrium turbinatum........	6.8	....
Mnium cuspidatum...............	5.2–8.0	5.6–8.0
Fissidens taxifolius...............	7.4	....
Brachythecium oxycladon..........	5.6–8.0	5.6–7.4
Brachythecium rutabulum.........	7.0–8.0	7.0
Anomodon apiculatus.............	7.2	....
Anomodon attenuatus.............	7.2–8.0	....
Amblystegium varium.............	5.2–8.0	....
Amblystegium serpens.............	7.4–8.0	....
Plagiothecium turfaceum.	7.2	....
Entodon cladorrhizans...........	6.8	....

DEVIL'S BACKBONE

The Devil's Backbone is a prominent ridge of St. Peters sandstone about 2 miles south of Oregon. Back from the road a short distance the ridge is topped by Platteville limestone, but near the road this has eroded leaving the friable sandstone exposed. This is rough and broken, forming many shelves, crevices, and flats for the growth of plants. Such places are filled with sand and small amounts of humus left from plant débris. As is the case in most conditions of this type, the south slope is xerophytic and the north more nearly mesophytic. The talus on the south side is almost pure sand, but on the north it is covered with a layer of humus formed from fallen leaves and other forest materials. Above the talus on both sides the rock is precipitous. It was this part of the ridge that gave most of the data for this work. The common species able to maintain a foothold on these ledges are *Vaccinium vacillans, Amelanchier canadensis, Rumex acetosella,* and a few scattered grasses and sedges. On the north side, where there is more moisture and shade, *Polypodium vulgare* has found lodging in the crevices.

The mosses found on this hill are typical of this kind of place. *Polytrichum commune, P. piliferum, P. ohioense, Catharinea angus-*

tata, Ceratodon purpureus, Dicranum scoparium, Dicranella hetero-malla, Leucobryum glaucum, Pohlia nutans, Bryum caespiticium, and *Plagiothecium pulchellum* have become established in various spots over the surface. On the south side they have formed mats on the thin sand covering the ledges. Growth is stunted and only a few plants produce any fruiting stalks. At the top of the highest part of the ridge the soil is so thin and the exposure so complete that *P. piliferum* alone has been able to maintain itself to any noticeable extent. It is mixed with *Cladonia sylvatica* and *C. pyxidata*. The mosses on

TABLE VII

DEVIL'S BACKBONE AND SINNISSIPPI KNOB; TESTS OF MOSSES AND TOP SOIL

SPECIES	RANGE OF pH																
	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0
Polytrichum commune			I	4	2	I		2	I	2	I		I	I			
Polytrichum piliferum	I		2	I	2	I	I	I	2	I	I						
Polytrichum ohioense		I	I														
Catharinea angustata					I		I		3	3							
Hedwigia albicans								2	I				I				
Ceratodon purpureus						2		I	2				I		I	I	
Dicranum scoparium	I		2		3			4	I	I							
Ditrichum pallidum									I								
Dicranella heteromalla	I		2			I		3	I								
Leucobryum glaucum					I	3											
Bryum caespiticium			2		2				I						I		
Pohlia nutans	I	I	I	I	5		I		2	2	I						
Brachythecium rutabulum														I			
Calliergon schreberi										I							
Plagiothecium pulchellum		I	2		I												

the north side are not any more numerous, but make a much more prolific growth than on other parts of the hill. They grow on ledges, in crevices, and on the vertical rock face without difficulty, and are able to fruit in season.

This hill was highly acid in all the places tested. The pH range for the mosses and the soil in their rhizoids was 3.8–5.4 (table VII). Readings of the sand not deeper than 1 inch ran 3.8–4.6. The adjustment of the moss flora on this rock was the closest found anywhere in the region. *Polytrichum ohioense* was found on the moist north slope. The other two species of *Polytrichum* were in drier places but in the same type of soil. *Plagiothecium pulchellum* grew well on the

acid rock in rather dry but shady spots. *Dicranella heteromalla*, *Dicranum scoparium*, and *Pohlia nutans* thrived on all sides but did best on the wetter face. Although the amount of water had much to do with the growth of the moss mat, it did not make much difference in the H-ion concentration of either the soils or the mosses.

SINNISSIPPI KNOBS

A few rods south of the Devil's Backbone are two prominent Knobs. These are of St. Peter sandstone with low and well rounded tops. The slopes are gentle and support a moderate mesophytic plant cover. There are some barren rock exposures at the top, but the surface is broad and is covered with a thin layer of soil. Trees of the oak-hickory climax reach almost to the tops and give a medium shade on the east, north, and west sides. The best growth of mosses is on these three sides and the tops.

The bases of the east slopes of these two hills were much alike and had similar moss floras. The common mosses were *Ceratodon purpureus*, *Funaria hygrometrica*, *Bryum caespiticium*, *Brachythecium rutabulum*, and *Amblystegiella adnata*. The mosses gave pH readings from 5.2 to 6.6 while the soil tests ran 4.8–5.6. These forms were growing well but did not develop into large thick mats. From half way up to the tops the moss species changed noticeably. Approaching the summits *Dicranella heteromalla*, *Bryum caespiticium*, *Catharinea angustata*, *Polytrichum commune*, *Pohlia nutans*, *Hedwigia albicans*, *Entodon seductrix*, and *Calliergon schreberi* appeared in well developed plots. *Dicranum scoparium* and *P. piliferum* occupied the higher places at the top. The pH tests of this group showed higher acid conditions than near the bases. The range for the moss materials was between 4.2 and 6.4 and the soil ran 4.2–5.6. In one case *Entodon seductrix*, growing near the top on rock, tested 6.8 for both the moss and thin layer of soil, but in most of the tests the pH stood about 5.2 and 5.4.

While the H-ion concentration at the bases of the slope approached close to the neutral point, the upper parts of the hills showed the usual acid reactions for the sand exposures of the region. Again it may be noted that it made little difference regarding the moisture content of the substratum. Tests from moss plots on bare rock ran

much like those on the sand and humus soil which held more moisture.

In the surrounding woods were many excellent moss plots. Growing on the rocks and sand were *Polytrichum commune, P. piliferum, Hedwigia albicans, Dicranum scoparium, Ditrichum pallidum, Ceratodon purpureus*, and a few other scattered species. Although the plots were not large, growth was good and sporophytes were usually present. The pH range of the moss material was 5.2–6.8 and that of the soil 4.4–5.6. A few tree mosses gave a different reaction. They consisted of *Pylaisia schimperi, Brachythecium rutabulum, Amblystegiella adnata*, and *Anomodon attenuatus*. Tests of this group ran between 5.6 and 7.4 with the majority between 6.8 and 7.2. Although these were miscellaneous spots, the mosses occupying the different places were very true to type.

Castle Rock and Vicinity

No other spot along the Black Hawk trail is more widely known and more frequently visited than the sandstone prominence about 3 miles south of Oregon called Castle Rock. It stands 100 feet or more above the river. The top is hard sandstone but the base on three sides is well built up from eroded sand which gives a good place for the growth of plants. The north side, being better shaded, supports some excellent beds of mosses. Characteristic plants of this slope are *Quercus alba, Q. ellipsoidalis, Amelanchier canadensis, Rubus alleghaniensis, Vaccinium vacillans, Maianthemum canadense, Polygonatum biflorum, Smilacina racemosa*, and *Dicentra cucullaria*.

In the vicinity of Castle Rock are five other hillsides upon which are found excellent moss plots. Three of them are south slopes and the other two are north. All are St. Peter sandstone outcrops, usually supporting the mesophytic vegetation commonly found in the oak woods of this region. The substrata are of sandy soil, generally mixed with humus of various depths, none exceeding 4 inches, and rock faces that are kept moist throughout most of the year by precipitation and seepage. All these places are well shaded during the growing season, either on account of the position of the hillside or by trees.

Mosses grew in great profusion in these several places. They occu-

pied rock ledges, shallow caves, vertical faces, moist and arid spots, and places of both shade and open sunlight. In many places they grew in such dense mats that they not only added humus to the soil but prevented erosion of the loose sand. The heaviest mats were of *Polytrichum commune, P. piliferum, Dicranum scoparium, Dicranella heteromalla, Catharinea angustata,* and *Calliergon schreberi.* In one place dead stems of *P. commune* extended more than 4 inches below the present level of the soil.

The mosses commonly found in this region are *Polytrichum commune, P. piliferum, Catharinea angustata, Dicranum scoparium, Ditrichum pallidum, Dicranella heteromalla, Pohlia nutans, Funaria hygrometrica, Georgia pellucida, Ceratodon purpureus, Amblystegiella adnata, Calliergon schreberi, Brachythecium rutabulum,* and *Mnium cuspidatum.* Others occurred less frequently and seemed to be out of their range, such as *Brachythecium oxycladon, Plagiothecium denticulatum, Anomodon attenuatus,* and *Bartramia pomiformis.* Even though the latter group was out of its natural habitat, the individual species maintained their usual reactions about the neutral point, 6.6–7.4. *P. commune* growing among these mosses also tested as high as 6.8.

The H-ion concentration of this whole group of moss habitats is consistently high, scarcely going below 5.6 in the soil tests at the 1 inch level. In a few cases the top soil with humus and rhizoids ran to 6.4–6.6. The pH readings for the mosses correlated closely with those of the soils, ranging from 4.2 to 6.8 (table VIII). This correlation, together with the fact that the mosses grew so vigorously and produced sporophytes, indicated strongly that so far as the moss flora of this region was concerned it was a climax situation. The few neutral and alkaline species found were not showing the vigor and growth noticed in the other group. Their present condition suggested that they were probably remnants of an earlier moss flora of a different type.

Species range

The preceding observations give some idea of the H-ion concentration of each of the different areas tested, but in no case were enough tests made of the individual species to be more than suggestive. The number of tests ranged from one for several species to

96 for *Polytrichum commune*. There was no special effort to favor any particular moss, but the number of tests indicates in a good measure the relative importance of each one in the Grand de Tour region. In this respect *P. commune* stands far above the rest, having occurred in every area where there was a sandy soil base.

Consideration of the species range requires a great number of pH readings to reach any definite conclusions. Since this work was not directed specially toward that problem, but to a more general pur-

TABLE VIII

SPECIES	RANGE OF pH	
	Moss tests	Tests of soil below surface
Polytrichum commune............	4.2–6.8	3.8–6.4
Polytrichum piliferum.............	4.4–6.8	4.2–6.8
Catharinea undulata.............	6.6	4.6
Catharinea angustata.............	4.4–6.2	3.8–5.4
Georgia pellucida................	3.8–5.6	4.2–5.2
Ceratodon purpureus.............	4.8–6.4	4.4–5.8
Dicranum scoparium.............	4.0–5.6	4.0–6.4
Ditrichum pallidum..............	4.4	4.2
Ditrichum tortile................	4.2	....
Dicranella heteromalla............	4.4–7.0	3.8–6.0
Pohlia nutans...................	3.8–5.2	3.8–5.0
Bryum caespiticium..............	4.8–6.8	5.4
Mnium cuspidatum...............	5.0–5.8	....
Anomodon rostratus.............	7.4	4.6
Anomodon attenuatus............	6.2–7.0	5.2
Brachythecium oxycladon.........	6.4–6.8	4.4–5.2
Brachythecium rutabulum........	7.0–7.2	5.2
Amblystegiella adnata............	4.6–4.8	....
Calliergon schreberi.............	4.8–7.2	4.2–7.4
Plagiothecium denticulatum.......	4.8–5.8	4.2–5.4
Hypnum haldanianum...........	4.0–5.0	4.0

pose, it is not expected that these figures should be considered final. Altogether there were 62 species of mosses found in this territory, belonging to 14 different families. Of these only 20 species showed tests of 10 or more, while 9 had 20 or more. A few of the outstanding ones will be mentioned merely for the purpose of emphasis.

All species of *Polytrichum* were acid and grew on acid soils. *Catharinea undulata* preferred soil from neutral to low acid, while the moss and top soil materials ranged from 5.4 to 8.2 with the majority of tests about neutral. *C. angustata* on the other hand was found in more acid soil and had a higher H-ion concentration than its related

species. *Pohlia nutans*, *Dicranum scoparium*, and *Dicranella hetero-malla* also grew well in acid soils. Two pleurocarps showed a preference for acid soils, *Calliergon schreberi* and *Hypnum haldanianium*. The outstanding mosses that ran from about the neutral point to the alkaline end of the scale were *Mnium cuspidatum*, *Bartramia pomiformis*, *Aulacomnium heterostichum*, *Brachythecium* spp., *Anomodon* spp., *Thuidium recognitum*, and *Climacium americanum*. Ceratodon

TABLE IX

RANGE OF pH OF MORE COMMON SPECIES FOUND IN REGION

SPECIES	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0	8.2
Polytrichum commune			1	2	5	3	10	14	8	11	3	4	5	5	6	11	1	1	3	1			
Polytrichum piliferum			2		3	2	2	6	7	6	1		2				3		1				
Catharinea undulata		1						1	1		2	3	5	5	2	6	5	4	5				
Catharinea angustata				1	1	1	4	6	6	6		3	2	3	1	2			1	1			
Dicranum scoparium	1	1	2	1	2		1	10	4	5		1			1								
Dicranella heteromalla	2		2	4	1	1		3	3	1		1			1								
Pohlia nutans	1	1	3	3	6	3	2	11	4	4	2	1											
Ceratodon purpureus					4	1		1	3			2	1	1	1			3	1				
Georgia pellucida	1		2	1	1	2	2	2	2														
Bryum caespiticium			2		2	1	1	2				1		2			1						
Bartramia pomiformis								1			2		1		1	1	5	10	3	3	4		
Aulacomnium heterostichum												1		2	1	5	3	4	1				
Mnium cuspidatum					1	1	2	1	1	2	1		2	2	1	1	9	9	4	3	2	2	
Thuidium recognitum								1			2	2	2		3	1		2	2	1			
Brachythecium oxycladon					1							1					1	1	3	2		2	2
Brachythecium rutabulum					1				1	1	1		1	4	1	2	1	2	4	1	3	3	
Calliergon schreberi						1				2	1	1				4							

purpureus although found on acid soils had a rather well distributed range, 4.6–7.6.

Another significant fact shown by these data was that the soil on which the mosses grew usually tested a little higher acid than the moss materials and top soils. The soils were not more constant at the 0.5–1 inch depth than the mosses, but seemed to vary rather closely with them. These two points seemed to be suggested, namely, the more acid the moss the more acid can be the soil on which it may grow; and the more alkaline the moss the less acid the soil which it will tolerate. Table IX gives the pH range for the mosses

that occurred most frequently. A list of mosses occurring in the region so few times that they were not charted is given in table X.

TABLE X

Moss	Num-ber of tests	pH range	Moss	Num-ber of tests	pH range
Polytrichum ohioense......	3	4.0–4.2	Anomodon minor.........	1	7.4
Polytrichum juniperinum...	2	5.6–5.8	Anomodon attenuatus.....	12	6.8–8.2
Catharinea crispa.........	1	7.6	Anomodon rostratus.......	5	7.4–7.8
Fissidens taxifolius........	1	7.4	Anomodon apiculatus......	1	7.0
Fissidens adiantoides......	1	7.0	Thelia lescurii............	2	7.0–7.2
Ditrichum pallidum.......	5	4.8–6.4	Leskia gracilescens........	1	7.6
Dicranum flagellare.......	1	4.6	Hylocomium triquetrum....	3	6.6–7.2
Dicranum pallidum........	3	5.0–5.2	Eurhynchium hians........	1	7.6
Leucobryum glaucum......	4	4.6–5.0	Eurhynchium serrulatum...	12	7.0–8.2
Grimmia apocarpa........	4	7.4–8.2	Brachythecium salebrosum.	3	5.4–7.0
Hedwigia albicans.........	5	5.4–6.2	Amblystegium serpens.....	10	5.8–8.2
Tortula ruralis............	1	7.8	Amblystegium varium.....	12	5.2–8.2
Barbula unguiculata.......	2	7.4–8.2	Amblystegium adnatum....	2	7.2
Encalypta ciliata.........	2	7.6	Climacium americanum.....	8	6.6–7.8
Funaria hygrometrica......	2	5.2–7.6	Hypnum haldanianum	5	3.8–5.4
Physcomitrium turbinatum.	2	6.0–6.6	Plagiothecium pulchellum..	4	4.0–6.0
Timmia cucullata	4	8.2	Plagiothecium turfaceum...	3	7.4
Bryum argenteum.........	4	6.2–8.2	Plagiothecium denticulatum	4	4.8–6.8
Bryum roseum............	3	6.2–7.6	Amblystegiella adnata.....	7	4.6–7.2
Bryum binum............	3	4.8–5.0	Entodon seductrix.........	7	6.8–7.6
Mnium hornum...........	2	7.0–7.4	Entodon cladorrhizans.....	3	6.8–7.4
Mnium stellare............	6	5.8–8.2	Pylaisia schimperi.........	5	5.6–7.4
Mnium spinulosum........	2	6.4–7.0			

Summary and conclusions

1. Mosses seem to have an H-ion range within which they can grow. Within these limits the optimum is usually near the median.

2. Mosses on the sandy soils of this region are generally the acid-loving types, although some are alkaline.

3. On the hilltops and on sandstone the mosses are acid-loving; at the bases of the hills and on the sides they may be alkaline.

4. Tree mosses run from neutral to alkaline; those on old wood are acid.

5. Mosses on limestone and the thin soils overlying it are alkaline; those on the banks of the small streams are also alkaline.

6. Most acid-loving mosses are acrocarps; most alkaline mosses are pleurocarps.

7. There is a close correlation of pH between the mosses and the substratum on which they are attached; there is some correlation between the mosses and the deeper soil, but not so close as with the former.

8. Moisture supply is an important factor in moss growth even when the H-ion concentration is suitable.

9. Mosses in mixed groups have a similar pH adjustment.

10. There are some indications that the mosses in this region have followed in successions.

11. Soil tests conform to the usual type. Hilltops and upper flats are acid while lower slopes and bases are neutral to alkaline.

12. Mosses have adjusted themselves to these conditions rather closely. They seem to have the same general relations to their substratum, so far as H-ion concentration is concerned, as the plants of the higher orders.

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ANTHERIDIAL DEHISCENCE IN THE POLYPODIACEAE

M. ELIZABETH HARTMAN

(WITH TWENTY-SEVEN FIGURES)

Introduction

Antheridial dehiscence in ferns has been under consideration since NÄGELI (23) in 1844 discovered the true antheridia. Various points of view concerning the dehiscence have been advanced, and taxonomic significance has been assigned to it. Separation of the Cyatheaceae and the Polypodiaceae partially on the basis of method of antheridial dehiscence is a striking example. In the Cyatheaceae the opercular cell was known to be extruded; in the Polypodiaceae it was believed to be ruptured. In 1911, however, SCHLUMBERGER (28) declared this distinction invalid, for he found extrusion of the cover cell to be characteristic of members of both families. His work, although accepted by GOEBEL (13), was not wholly accepted by English and American investigators. Further investigation of antheridial dehiscence in the Polypodiaceae was considered desirable, therefore, and these studies were begun in 1925. The morphological aspect of the problem was first considered and investigation of living material made. Microchemical studies were later attempted, in an effort to explain more fully the mechanism of dehiscence. These two phases will be treated separately. The following historical summary indicates the present status of the problem.

Materials and methods

The general method employed in this investigation was direct microscopic observation of living material mounted in distilled water; in addition, a few prepared slides were made. The photomicrographs were made with a Bausch and Lomb apparatus from living specimens, and a camera lucida was employed in drawing. For most of the work a no. 10 ocular and 4 mm. objective were used.

The cultures were grown in covered glass vessels kept on a laboratory table by a north window, with the same side of the dish always

HISTORICAL SUMMARY

DATE	INVESTIGATOR	MATERIAL	INTERPRETATION OF DEHISCENCE
1844	Nägeli (23)	Over 100 species from botanical garden at Zürich, grown for germination studies; figures Aspidium augescens, A. concinnum, Asplenium dissectum	Antheridium splits at summit. Whether mature or not, if brought into contact with water it discharges contents.
1848	Leszczyc-Suminski (20)	Various species, particularly Pteris serrulata	Spontaneous apical rupture at maturity.
1849	Wigand (37)	Various species growing on flower pots, including Adiantum pubescens, A. capillus-veneris (?), Pteris sp., Aspidium capense, etc.	Star-shaped apical rupture through pressure or spontaneously at maturity. Agency of water seems unessential.
1849	Thuret (32)	Various species; figures Scolopendrium officinale and Pteris aquilina	Apical cell ruptured or sometimes discharged through hole in "cuticle" by pressure of enlarging spermatogenous mass; process comparable with that in mosses. Subsequent intrusion of ring cell.
1849	Schacht (27)	Pteris serrulata, Asplenium petrarcae, Adiantum formosum, Aspidium violaceum	Cover cell gradually lifts itself more or less completely from other cells, but also frequently seems to burst. State of ripeness, rather than presence of water, apparently essential factor.
1862	Hofmeister (17)	General; figures Pteris serrulata for antheridia	If ripe antheridium brought into contact with moisture, its contents swell and apical cell bursts in stellate manner.
1869–1870	Strasburger (29)	Pteris serrulata (and Ceratopteris thalictroides)	Star-shaped rupture of cap cell results from pressure of interior, if ripe antheridium brought into contact with water.
1869	Kny (19)	Asplenium alatum, also non-polypod species including Cibotium schiedei	Cap cell at maturity irregularly ruptured, probably through turgescence of ring and basal cells. Folds in ring cell noted (cap cell detached in Cibotium schiedei).
1878	Bauke (2)	Pteris aquilina, P. cretica, cyatheoid species	Irregular rupture of cover cell in Polypodiaceae due to tension of peripheral cells and swelling of spermatogenous mass. Water important. Extrusion of cap cell characteristic in Cyatheaceae.

HISTORICAL SUMMARY—*Continued*

DATE	INVESTIGATOR	MATERIAL	INTERPRETATION OF DEHISCENCE
1896	Heim (16)	Doodia caudata, etc.	Undivided cap cell irregularly ruptured at maturity in Polypodiaceae through turgescence of ring cells.
1898–1901	Goebel (12)	General	Rupture of cover cells characteristic of the Polypodiaceae, Aneimia, and Mohria; details of mechanism unknown.
1902	Britton and Taylor (5)	Vittaria lineata	Cap cell ruptured centrally or laterally.
1908	Conard (9)	Dicksonia punctilobula	Cap cell ruptured irregularly; contents of antheridium swell by absorption of water.
1911	Schlumberger (28)	Cibotium schiedei, Hemitelia aspera, Cyathea dealbata, Diacalpe aspidioides, Woodsia obtusa, W. ilvensis, W. hyperborea, Cystopteris fragilis, Athyrium filix-femina, Asplenium ruta muraria, Scolopendrium officinale, Pteris serrulata, Polypodium aureum	Normal method of opening for polypod antheridium is detachment of cap cell; rupture absent. Water essential and wall cells take active part in opening.
1918	Campbell (7)	General; Onoclea struthiopteris used as type	Cap cell ruptured or less commonly extruded.
1918	Goebel (13)	General (follows Schlumberger)	Wall cells active in opening of antheridium in pteridophytes, as in bryophytes. Within the pteridophytes antheridia differ as to number of cells of apical layer taking direct part in opening. Detachment of one opercular cell is regular in leptosporangiates (including Polypodiaceae).
1921	Strasburger (30) (and others)	General	Cap cell ruptured by pressure of swollen ring cells. (This edition of textbook makes use of Schlumberger's figures of extruded cap cell.)
1923	Bower (3)	Leptosporangiate ferns in general	Cites Campbell, Goebel (1918), and Schlumberger. Uses figures of Schlumberger.
1927	Pickett and Thayer (24)	Polypodium vulgare var. occidentale, Pellaea densa	Antheridia open by pore which is 4-sided in Pellaea densa.
1928	Bower (4)	Dryopteroid ferns	Further reference to Schlumberger's work.

toward the light. At Mount Holyoke the substratum used was peat; in Nebraska, sphagnum. For most of the cultures the dishes and substrata were boiled in water. This process was found preferable to sterilization in the autoclave. When dry, the cultures were watered with distilled water. A number of the cultures survived the summer vacation seasons with very little care. Forceps dipped in 95 per cent alcohol and flamed were used in lifting the gametophytes.

Potassium permanganate was used, as necessary, to check the growth of molds. This treatment, suggested by Dr. STOKEY, proved effective. A few small crystals of the permanganate were added to distilled water in a dropper bottle, and a small quantity of the deeply colored solution ("decidedly pink" or even purple) dropped upon the infected areas, the treatment being repeated as often as seemed necessary. Care was exercised to avoid an excess of water in the cultures.

Both fresh and dried spores of native and greenhouse species were planted as available, a total of 24 species being cultured. Lack of time prevented the study of some of the cultures. The spores of *Microlepia platyphylla* (later identified by Dr. W. R. MAXON) were brought by Dr. STOKEY from Allan Park, Toronto.

Morphological investigation

In the preliminary studies, which consisted of observations of the frequency of cap cell extrusion in the available polypod species, the results varied from those with antheridia of *Microlepia platyphylla* (fig. 1), where a detached cap cell could be found for nearly every freshly opened antheridium, to those of the related species *Davallia pentaphylla*, where the detached cap cell was never seen during this early work. The fate of the cap cell, when it was not visibly extruded, remained undetermined. Neither the theory of a slightly tilted, lidlike cap cell under which the sperm mother cells might pass (SCHLUMBERGER 28), nor the recognition of possible errors of observation seemed satisfactorily to account for the numerous failures to see the extruded cell.

The general conclusions from this unpublished work (1925) follow:

1. An extrusion of the cap cell, or its tilting, is indicated as the regular means of dehiscence for *Microlepia platyphylla*, *Dennstaedtia punctilobula* (both wild and pure), and *Aspidium marginale*.

2. An extrusion of the cap cell occurs also in *Polystichum acrostichoides* and *Woodsia ilvensis*.

3. It is probable that it may also be found in a wild hairy form, possibly *A. spinulosum*, and *Davallia pentaphylla*.

4. A rupture of the cap cell was never observed, although this alternative was not wholly disproved.

The morphological work was later continued (1926–1928) with more detailed study of the mechanism involved in the dehiscence, and increased effort to explain the instances when the detached cap cell failed to appear. Microchemical studies were also undertaken.

The changes in the ripe antheridium antecedent to opening were studied in greater detail, using greenhouse prothallia, probably *Aspidium simulatum*. Ripe antheridia were watched closely, the time period varying from a few minutes to three hours for an antheridium to open. The details observed were essentially those reported by earlier writers, including SCHLUMBERGER:

1. Gradual swelling of the spermatogenous mass.
2. Resulting compression of the peripheral cells: (a) imprint of the sperm mother cells first evident on the ring and cap cell and later on the funnel cell; (b) cap and ring cells sometimes scarcely perceptible; (c) funnel cell less completely compressed (this cell also differs from the others in stained sections, appearing redder with Flemming's triple stain).
3. Final opening instantaneous.
4. Quick inward protrusion of the previously distended peripheral cells, forcing out the sperm mother cells.
5. First ejectments very rapid.
6. Successive ejectments slower.

This process, with minor variations, seemed to characterize all species studied. As the swelling of the spermatogenous mass results in almost complete compression of the ring and cap cells and less compression of the basal cell, pressure of the swelling central mass together with that of the basal cell seems to be the primary agency in effecting the opening, while counter pressure of the ring and cap

cell seems to be secondary. A jerk immediately preceding dehiscence was noted with antheridia of *Polypodium iroides*, and was found to be a common accompaniment of the opening in various species. The compression of the peripheral cells before opening, in contrast with their expansion after, is illustrated in figs. 1–27.

Observations of the frequency of cap cell detachment were continued with variable results. Detached cap cells were now found for *Davallia pentaphylla*. With *Polypodium aureum*, however, actual extrusion was not observed. Sometimes this cell seemed to be in position, but tilted so as to permit the passage of the sperm mother cells. At other times, when a rupture seemed not improbable, folds in the ring cell were found to be responsible for the semblance of a star-shaped cleft, as explained by SCHLUMBERGER (figs. 2, 4, 5, 26). With *Aspidium marginale* detached cap cells frequently appeared. Antheridia of gametophytes from the drier situations in the culture vessels seemed to exhibit especially vigorous opening. Extrusion of the intact cap cell was occasionally observed with antheridia of *Woodwardia orientalis*. Tilted, lidlike cap cells were observed in *Adiantum pedatum;* many detached cap cells were observed in *Aspidium simulatum*, being seen readily even with low magnification. Detached opercular cells were also seen in *Polystichum acrostichoides*. Gametophytes of *Pellaea atropurpurea* showed opening antheridia, but no conclusive information concerning the dehiscence was obtained. In spite of this variability of results, there was no definite evidence of essential difference in antheridial dehiscence in the various species.

A study of the antheridia of *Adiantum pedatum* suggested the frailty of the detached cap cell as a factor in accounting for failures to see this cell after dehiscence. The detached cells seemed to disintegrate readily. In subsequent study of *Woodsia ilvensis* an extruded cell of very indistinct outline was seen. The dimness might have prevented its identification if the actual ejectment had not been observed. Two extruded cap cells of *Woodsia ilvensis*, however, observed later, seemed less evanescent. Of these, one was from the first antheridium to open in a group of twelve, simultaneously visible. Eight of the remaining eleven antheridia opened. They showed no detached cap cells after dehiscence, but only green granular mat-

ter at the apex. Because of the frequency of the appearance of this
granular material at the apex of open antheridia which failed to
show the detached opercular cell, and of the similarity of its green
granules to those seen in some of the detached cells, it seemed not
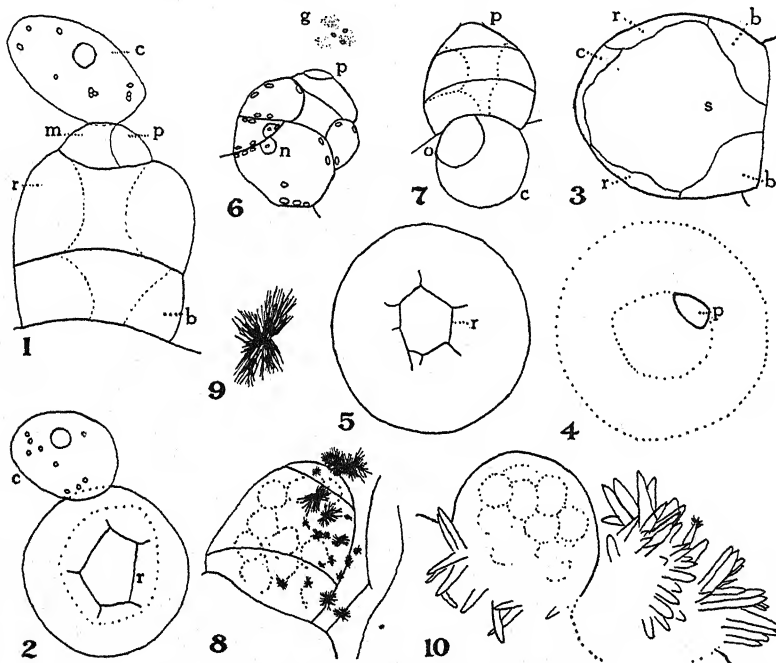
FIGS. 1–10.—Fig. 1, open antheridium of *Microlepia platyphylla*, lateral view; de-
tached cap cell (*c*) showing nucleus and chloroplasts; outer membrane (*m*); pore in outer
membrane (*p*); ring cell (*r*); basal cell (*b*); ×493. Fig. 2, *Adiantum pedatum*, apical
view of antheridium; cap cell (*c*) folds in ring cell; ×333. Fig. 3, mature unopened
antheridium of *Onoclea sensibilis*, optical section; spermatogenous mass (*s*); ×414.
Fig. 4, *O. sensibilis*, apical view; pore in outer membrane (*p*); inner and outer edge of
ring cell indicated with dotted lines; ×414. Fig. 5, same as fig. 4, lower level, showing
folds in ring cell; ×414. Fig. 6, antheridium of *O. sensibilis*; pore in outer membrane
(*p*); extruded granular mass (*g*); two nuclei of divided basal cell (*n*); ×293. Fig. 7,
adjacent antheridia of *Athyrium filix-foemina* showing diagrammatic lateral view of
normal antheridium with cap cell slightly projecting (*p*) and apical view of antheridium
with division of original cap cell; opercular daughter cell (*o*); lunate daughter cell (*c*);
×414. Fig. 8, antheridium of *O. sensibilis* with glucosazone clusters on and near
antheridium; ×414. Fig. 9, same as fig. 8; larger glucosazone cluster from field; ×414.
Fig. 10, maltosazones massed about two antheridia of *O sensibilis;* antheridium on
right almost covered by crystals; ×414.

improbable that the green slime resulted from disintegration of the cap cell before or after dehiscence.

The occasional frailty of the cap cell was also observed with *Onoclea sensibilis.* At first detached cells were found with difficulty.
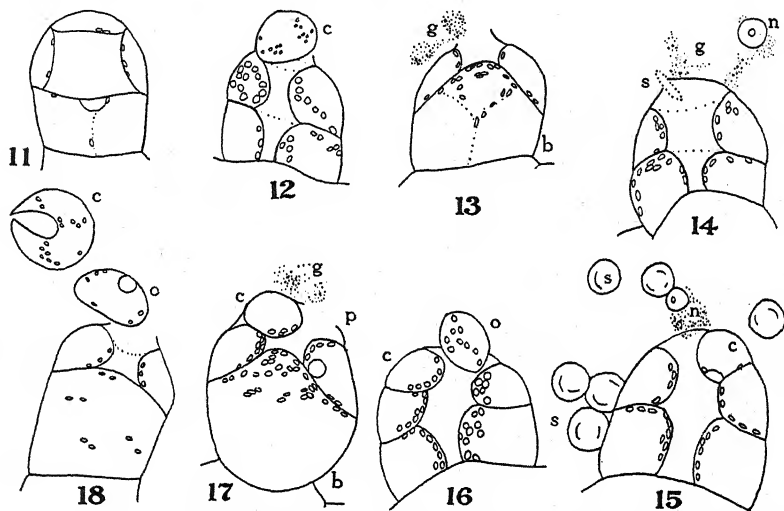
FIGS. 11–18.—*Woodsia obtusa:* Fig. 11, young antheridium. Fig. 12, antheridium with undivided cap cell partially extruded; pore in outer membrane visible. Fig. 13, antheridium with extruded content of undivided cap cell (pore in outer membrane evident, note swollen basal cell). Fig. 14, antheridium with extruded cap cell content containing nucleus with nucleolus; dotted ellipse represents elongated sperm mother cell (*s*) passing through pore in outer membrane. Fig. 15, antheridium with extruded content of opercular daughter cell, including nucleus, lunate daughter cell in place, and spermatocytes (*s*). Fig. 16, antheridium with divided cap cell; opercular daughter cell partially extruded; lunate daughter cell in position. Fig. 17, antheridium with extruded content of opercular daughter cell (nucleus in ring cell). Fig. 18, antheridium with divided cap cell; both daughter cells extruded (*o, c*) (nucleus visible in smaller daughter cell); ×293.

One became evident after the mass of sperm mother cells had scattered from the mouth of an antheridium. This cell, also, seemed evanescent: after 15 minutes it was too indistinct for identification, had its position been unknown. The wall seemed to dissolve in water. Numerous additional detached cells were found, some of which were in good condition 15 hours after extrusion. Most of these
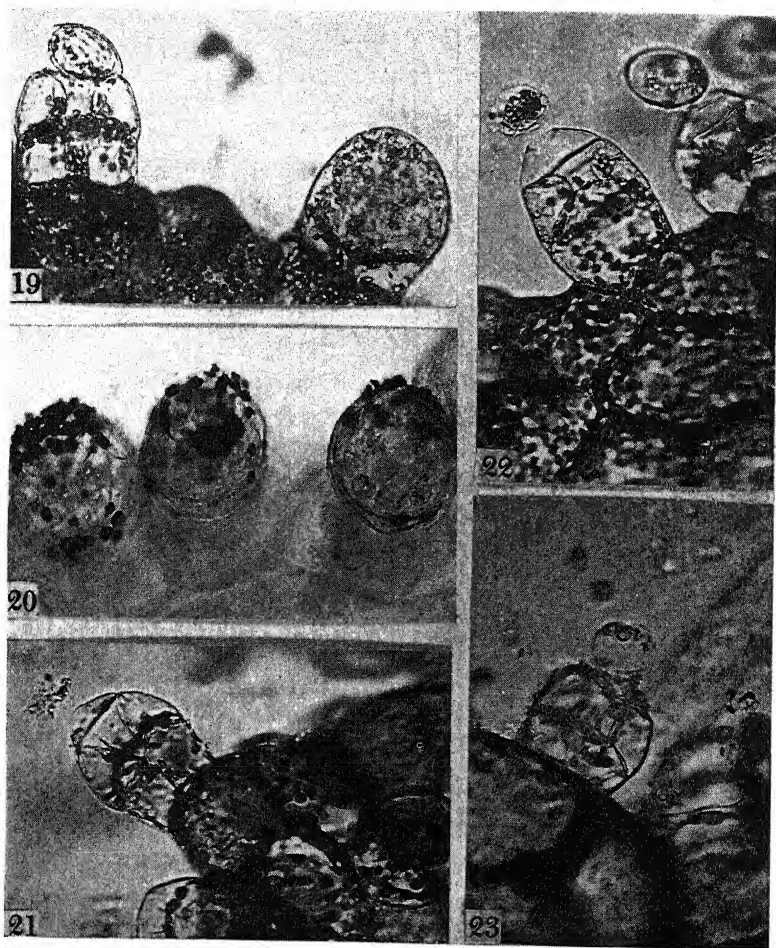
FIGS. 19–23.—Fig. 19, *Onoclea sensibilis* showing open antheridium with tilted cap cell under which sperm mother cells passed (left) and mature unopened antheridium at right (note compression of wall cells before dehiscence and expansion after dehiscence). Fig. 20, *O. sensibilis;* antheridia from sugar test (Flückiger's) with crystals of cuprous oxide especially abundant at apex. Fig. 21, *Athyrium filix-foemina;* lateral view of open antheridium with extruded granular mass of cap cell content. Fig. 22, *A. filix-foemina*, showing opened antheridium with pore in outer membrane and detached cap cell with partially disintegrated content (right), and another detached cap cell. Fig. 23, *A. filix-foemina;* antheridium with detached cap cell showing nucleus; sperms and rhizoid visible in field; ×350.
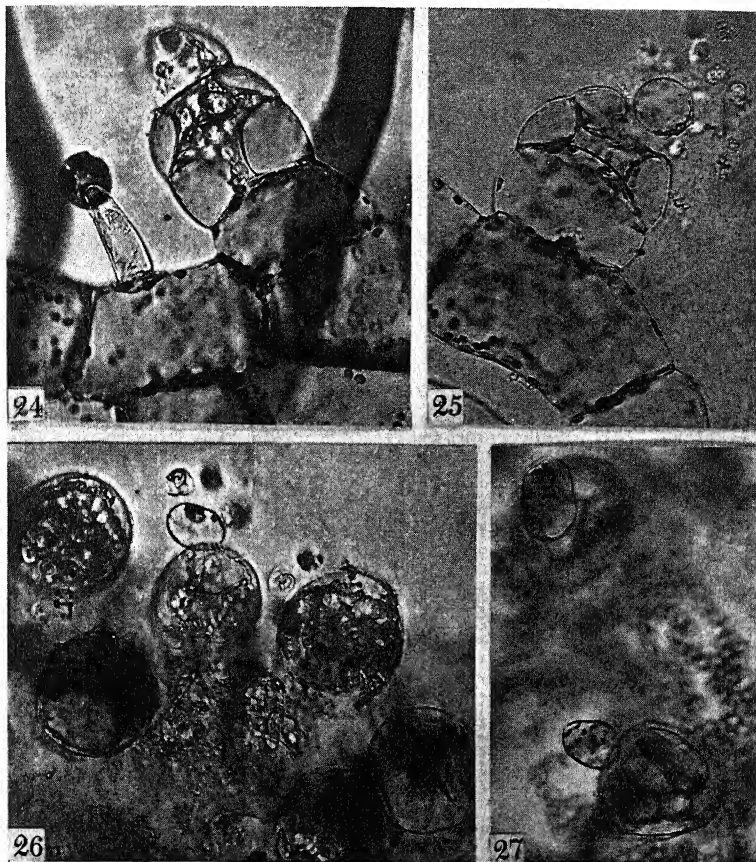
FIGS. 24–27.—Fig. 24, *Woodsia obtusa;* open antheridium with undivided cap cell (out of focus and appearing as shadow); sperms within antheridium; glandular hair at left of antheridium; rhizoid as long shadow at right. Fig. 25, *W. obtusa;* filamentous thallus showing open antheridium with divided cap cell; opercular daughter cell detached; its sister cell in place; sperms about antheridium (cf. fig. 16). Fig. 26, *A. filix-foemina;* apical view of open antheridium with detached cap cell; unopened antheridia; old opened antheridium showing darkened cavity surrounded by folded ring cell; sperms in field. Fig. 27, *Alsophila cooperi;* (below) apical view of antheridium with divided cap cell, opercular cell extruded; (above) apical view of another antheridium with detached cap cell; ×350.

showed well defined wall, nucleus, and chloroplasts, although a few seemed to contain only small green granules (sometimes in active Brownian movement), and one or two, lying against the green thallus, were so indistinct that it is questionable whether an intact extruded cap cell or merely the green slime was seen. With lateral views of the antheridium the hole in the outer membrane was visible (figs. 1, 6, 12–14, 17–19, 22); with apical views the folds in the ring cell were conspicuous and the pore in the outer membrane could be seen at a higher level (figs. 4, 5). A striking example of the extrusion of the sperm mother cells under a tilted cap was also seen. The first sperm mother cells passed out under the cap cell (fig. 19), but successive ejectments gradually loosened it until it became wholly extruded. At other times all the exits were made under a tilted cap cell (figs. 7 *p*, 19); in some cases this cell was seen to be lifted by each escaping sperm mother cell and then to slip into position again. With *Cyrtomium falcatum* similar types of dehiscence were found, and also a resistant cap cell which withstood 10 minutes of heating on the water bath with acidified phloroglucinol.

Variability in the number of peripheral cells is a new feature found in antheridia of *Athyrium filix-foemina*. About 12 antheridia, each with a divided cap cell, were seen (fig. 7 *o*, *c*), and several antheridia with two ring cells in addition to the basal cell. Cap cell detachment was especially frequent and typical.

The occasional division of the cap cell in *Athyrium filix-foemina* is of interest. Both SCHLUMBERGER (28) and GOEBEL (13) state that it regularly remains undivided in the Polypodiaceae, and BOWER (4) records this opinion. Of the polypod genera previously investigated, only *Diacalpe* and *Woodsia* showed division. In *D. aspidioides* this division (according to SCHLUMBERGER) is strictly of the cyatheoid type, being accomplished by a transverse curved wall, sometimes followed by a second wall perpendicular to the first. In *W. obtusa* the division differs in that a definitely circular daughter cell is formed to one side of the original cap cell, and no further divisions occur. As division is frequent in *W. obtusa* and rare in *W. ilvensis*, this genus has been regarded as transitional between the Cyatheaceae and the Polypodiaceae.

In this study of the polypod antheridium, the division of the cap

cell was noted in *Woodsia obtusa* and *Athyrium filix-foemina*. These observations (figs. 7, 15–18, 25) may be compared with the cyatheoid type of division, as illustrated by *Alsophila cooperi* in fig. 27. In this connection it is of interest to note that both CHRISTENSEN (8) and WETTSTEIN (36) place the Woodsieae next to the Cyatheaceae.

Detailed study of *Woodsia obtusa* followed. Although both divided and undivided cap cells were found, the smaller daughter cell was often not evident before dehiscence because of its position and the compression due to the swelling of the spermatogenous mass. When the cap cell was divided, each daughter cell had a nucleus. In case of the undivided cap cell, the intact detached cap was sometimes found (figs. 12 *c*, 24) as in other species. When there were two cap cells, the smaller daughter cell was frequently extruded (figs. 16 *o*, 25). In one case both daughter cells were extruded, for two detached cells (one lunate and one ovoid) appeared before one open antheridium (fig. 18 *c*, *o*). Some open antheridia failed to show detached cells, but instead granular material appeared at the apex (figs. 13, 17). This slime, like that of other species, contained small green granules suggestive of the decomposing chloroplasts of the extruded cap cell.

The actual relation of this granular material to the cap cell seemed to be established when a nucleus with nucleolus was observed lying in the green slime (fig. 14 *n*). A second nucleus was seen in the material from another antheridium (fig. 15 *n*), this having a divided cap cell. Later nuclei were observed definitely in the extruded granular mass of other species, one from an antheridium of *Onoclea sensibilis*, and a few from antheridia of *Athyrium filix-foemina*.

Further to verify the idea that the green granular material is probably the content of the cap cell, additional antheridia were studied. With one antheridium of *Woodsia obtusa* it was definitely seen that the greenish granular slime preceded the spermatogenous mass. With *Cyrtomium falcatum* this priority of appearance was more readily observed, as the dehiscence is less nearly instantaneous. *Woodwardia angustifolia* also showed material appearing before the sperm mother cells, as does the detached cap cell. The hole in the outer membrane was seen distinctly.

The fact that the nucleus is not always seen with the green granules needs explanation, but does not necessarily discredit the theory

that these granules represent cap cell content. The nucleus may possibly disintegrate, as some of the observations seemed to show, or it may be hidden by the green granules. Being uncolored, it is frequently indistinct. A comparison of its visibility in extruded cap cells seemed significant. Of two detached cells lying side by side in *Athyrium filix-foemina*, one showed clearly defined wall, chloroplasts, and nucleus; the other less distinct wall and scattered green granules without well defined chloroplasts or nucleus. Of six detached cap cells from six successive marginal antheridia of the same slide, three showed the nucleus clearly and three failed to show it. Of three others chosen for the sharp definition of the cap cells, the nucleus could be identified in all. A fourth successive antheridium showed the green granular mass with a nucleus but no cell wall. This fourth antheridium seemed to have a lunate cap cell in place, thus providing another example of a divided cap cell seen in *A. filix-foemina*.

It is therefore apparent that the distinctness, as well as the condition, of the detached cap cells varies. The observed range of possibilities is illustrated in the photomicrographs of *Athyrium filix-foemina*. Figs. 23 and 26 show typical detached cap cells in good condition. The nucleus is especially distinct in fig. 23. In fig. 22 the detached cap cell is also typical, but the cell content is somewhat degenerated. As a third possibility, fig. 21 shows the extruded granular matter which represents cap cell content. The additional variation, that of the tilted or partially detached cap cell, is illustrated for *Onoclea sensibilis* in fig. 19. The pore or hole in the outer membrane through which the cap cell passes is evident in fig. 22.

A discussion of the microchemical investigations is to follow, but, summarizing the morphological work, the following facts have been observed:

I. The cap cell is regularly undivided in the polypod species studied, except *Woodsia obtusa*, where division is frequent, and *Athyrium filix-foemina*, where it is occasional.

II. Extrusion of the intact cap cell is a frequent method of antheridial dehiscence in many polypod species: *Onoclea sensibilis, Aspidium marginale, Aspidium simulatum, Polystichum acrostichoides, Athyrium filix-foemina, Woodsia obtusa, Dennstaedtia punctilobula, Microlepia platyphylla.*

Extrusion of the intact cap cell is an almost unvarying method of dehiscence in certain species, as *Athyrium filix-foemina* and *Microlepia platyphylla*.

III. When the cap cell is divided, extrusion of one of the daughter cells is usual, as in *Woodsia obtusa* and *Athyrium filix-foemina*.

IV. Extrusion of the intact cap cell occurs at least occasionally in other species: *Davallia pentaphylla, Adiantum pedatum, Woodsia ilvensis, Cyrtomium falcatum, Woodwardia orientalis*.

V. Tilting of the cap cell, sometimes found in place of complete extrusion, is equivalent to it and may become identical, as in *Onoclea sensibilis*.

VI. In a given species, the frequency of total extrusion of the intact cap cell may vary, apparently with physiological or cultural conditions, as observed in *Onoclea sensibilis* and others.

VII. In the absence of the intact detached cap cell, an extruded green granular mass is frequently seen, as in: *Polypodium aureum, Woodsia ilvensis, Onoclea sensibilis, Cyrtomium falcatum, Woodsia obtusa, Woodwardia angustifolia, Athyrium filix-foemina, Polystichum acrostichoides*.

VIII. This granular mass bears a striking resemblance to the content of the extruded cap cell, in that: (a) it contains green granular material analogous to the decomposing chloroplasts seen in some of the detached cells (*Woodsia ilvensis, Onoclea sensibilis, Athyrium filix-foemina*); (b) it sometimes contains a well defined nucleus (*Woodsia obtusa, Onoclea sensibilis, Athyrium filix-foemina*); (c) it is extruded before the spermatogenous mass (*Cyrtomium falcatum, Woodsia obtusa, Woodwardia angustifolia*).

IX. The nucleus may be invisible even in the intact extruded cell (*Athyrium filix-foemina*).

X. There is, regardless of cap cell behavior, a hole in the outer membrane of the antheridium, as seen in most species studied.

XI. Swelling of the spermatogenous mass is an important factor, and probably the primary agency, in causing rupture of the outer membrane, as seen especially in *Aspidium simulatum*.

XII. Intrusion of the ring and basal cell effects extrusion of the spermatocytes, as seen in all species.

These observed facts support the following conclusions:

1. Detachment of the intact cap cell (complete extrusion or mere tilting) is a general method of antheridial dehiscence in the Polypodiaceae.

2. Extrusion of green granular material represents extrusion of cap cell content.

3. These two types of dehiscence are therefore essentially one, namely, an extrusion of the opercular cell with or without the intact inner membrane.

4. A star-shaped rupture of the apical cell, in the sense of the earlier writers, does not occur.

5. The wall cells are active in antheridial dehiscence, although swelling of the spermatogenous mass seems to be primary in causing the initial opening: (a) the cap cell, or its daughter cell, forms at maturity a detachable operculum; (b) the basal cell aids in the pressure which causes rupture of the outer membrane; (c) the ring and basal cells, by their inward protrusion, eject the spermatogenous mass.

A reconstructed general view of antheridial dehiscence in the Polypodiaceae may then be given, as follows:

When the mature antheridium has access to water, the spermatogenous mass swells until its pressure, probably aided by that of the basal cell, causes rupture of the outer membrane (original wall) of the antheridium. The opercular cell, with or without its inner wall intact, is then extruded or sometimes merely tilted, and the spermatocytes follow, being pushed out chiefly by force of the intrusion of the ring and basal cells. The opercular cell is regularly the undivided cap cell; but frequently in *Woodsia obtusa*, and sometimes in *Athyrium filix-foemina*, it is a daughter cell of the original cap cell.

Microchemical investigations

The series of tests outlined by ECKERSON (10) were used for the microchemical studies. As occasion arose, this outline was supplemented by general works, such as those of TUNMANN (34), MOLISCH (22), HAAS AND HILL (14), THATCHER (31), ONSLOW (24), and HAWK (15), as well as by detailed research papers.

The tests were performed repeatedly, and were checked as far as

possible, either with the pure chemical or with plant parts known to contain the substance in question. Although this work is not exhaustive, interesting data are available.

The walls of the gametophyte, including those of the antheridia, are partly pectic in nature, as shown by the ruthenium red color test. For example, when gametophytes of *Athyrium filix-foemina* are mounted in water and then treated with ruthenium red, the following reaction is obtained:

A. VEGETATIVE PARTS:
  1. Rhizoids stain vividly.
  2. Prothallial cell walls stain.
     a. In the old degenerating portion near the spore.
     b. In the healthy tissue where a tear occurred.

B. ANTHERIDIAL PARTS:
  1. Freshly opened antheridia.
     a. Ring and basal cells become pink: (1) diffusely pink throughout; (2) deeper pink, frequently, along upper wall of funnel cell and inner and upper walls of ring cell.
     b. Detached cap cells stain: (1) sometimes more vividly than the other cells; (2) sometimes less vividly.
     c. Sperm mother cell membranes stain; the sperm within remains colorless.
     d. Vesicles of freed sperms may stain slightly.
     e. Nuclei sometimes stain in: (1) ring or basal cell; (2) detached cap cell; (3) granular mass of extruded cap cell contents.
  2. Old open antheridia.
     a. The central cavity stains vividly.
  3. Unopened antheridia.
     a. Usually stain slightly or not at all.
     b. If accidentally torn from the thallus, the spermatogenous mass stains vividly (detachment apparently favoring penetration of reagent).

Similar results were obtained with the other species studied, *Aspidium simulatum*, *Polypodium iroides*, *Davallia pentaphylla*, *Onoclea sensibilis*, and *Cyrtomium falcatum*, but the observations were not in every case as full as those just outlined. The test is less defi-

nite if gametophytes are mounted directly in a weak aqueous solution of ruthenium red, although the antheridia are capable of opening in this medium. In check tests with methylene blue only *Athyrium filix-foemina* and *Aspidium simulatum* were used.

Solubility tests, following ECKERSON (10), (cf. TIFFANY 32, HOWE 18, and ROBERTS 26), indicate that the walls probably contain pectose, as they fail to restrain after treatment with dilute hydrochloric acid. The pectic materials of the rhizoids, however, are often resistant to both acid and alkali. The sperm mother cell membranes seem to be pectin, as they dissolve in water. The nature of the pectic material of the cap cell remained undetermined, as the manipulations of the solubility tests repeatedly caused loss of this cell.

The swelling of a slime in which the sperm mother cells are imbedded has been suggested as a factor important in the opening (SCHLUMBERGER 28, HOFMEISTER 17, BAUKE 2). This study indicates that swelling of the spermatogenous mass results from the pectin of the sperm mother cell membranes. It may be that a pectose or pectinogen is converted by hydrolysis into pectin during the ripening process (cf. HAAS and HILL 14, ONSLOW 24, THATCHER 31 on pectic substances). Other factors, such as the possible presence of a trace of pectin from the dissolution of the middle lamella of the cap cell, may act in the mechanism. As to the swelling of the peripheral cells, it is not wholly certain whether the pectic materials found in them are identical with the hyaline slime found by SCHLUMBERGER (28) in his use of ruthenium red. This slime is also emphasized by GOEBEL (13) in explaining the activity of the wall cells.

The walls of the gametophyte, including those of the antheridia, are also partly cellulose in nature. In general, the usual cellulose tests were positive, although there was some variation in results. This variation probably was caused, in part at least, by the presence of other substances with the cellulose. With the usual iodine sulphuric acid color test, best results were obtained with the following modification of the procedure suggested by ECKERSON (10):

a. Limitation of the reaction period in the iodine potassium iodide solution to approximately 10 minutes.

b. Addition of a drop of water with or immediately following the

sulphuric acid (specimens mounted first in water were touched to filter paper before being transferred to a drop of the iodine solution).

The results were as follows:

A. VEGETATIVE PARTS:

    1. Prothallial cell walls become blue.

    2. Rhizoids become blue.

    3. Walls of prothallial hairs become blue.

B. ANTHERIDIAL PARTS:

    1. Antheridium in general becomes blue: (a) it may be bluer at base than at apex; (b) basal cell may be decidedly more blue than ring cell; (c) detached cap cell may shrivel or swell on addition of sulphuric acid (or shrivel and expand again); (d) detached cap cell fails to become blue, in one case it became slightly blue; (e) young antheridia fail to become blue, although adjacent prothallial walls are blue.

The following species were used in these tests: *Aspidium simulatum, Cyrtomium falcatum, Athyrium filix-foemina, Onoclea sensibilis, Polystichum acrostichoides*, and *Polystichum aculeatum*. Again the more detailed work was done with *Athyrium*. However, no consistent variation of reaction was found for any given species. The use of iodine-zinc chloride (HAWK **15**, after NOWOPOKROWSKY) was confirmatory of these results, in that antheridial and prothallial walls became blue in this reagent. Additional iodine potassium iodide was used, until the reagent would color tissue from *Capsella*.

The presence of cellulose was further verified by means of crystallization. With a gametophyte of *Athyrium filix-foemina*, cellulose crystals were obtained which compared favorably with those shown by MOLISCH (**22**). The crystals in the prothallial cells were more distinct than those about the antheridia.

Solubility tests were also made, and again *A. filix-foemina* was used. After several days in ammoniacal copper oxide, gametophytes stained a reddish blue when placed in the iodine reagent. This bluing seemed to involve cell contents rather than cell walls. Addition of sulphuric acid removed the blue. The residue from these tests gave a strong pectic reaction.

With polarized light, the presence of cellulose in gametophytes of *A. filix-foemina* was indicated as follows:

A. VEGETATIVE PARTS:
  1. Prothallial cell walls strongly anisotropic.
  2. Walls of rhizoids also anisotropic.
B. ANTHERIDIAL PARTS:
  1. Outer membrane of antheridium: (a) this wall is strongly aniso-
     tropic, even in young antheridia; (b) isotropic spermatocytes
     were seen passing out through the opening in this luminous
     membrane; (c) in only one case did this membrane appear to
     be lacking in cellulose (isotropic), and then merely in the ex-
     treme apical portion.
  2. Cap cell: (a) cap cell is frequently wholly isotropic; (b) in two
     cases, an extruded cap cell appeared to have a trace of cellulose
     at each end.
  3. Ring cell: (a) this cell varied in luminosity in different anther-
     idia, but appeared to contain relatively little cellulose.
  4. Basal cell: (a) basal cell also varied in luminosity, but fre-
     quently appeared to have more cellulose than ring cell; (b) in
     some cases it appeared to lack cellulose only in the central por-
     tion of its upper wall; (c) in one case the upper wall was lumi-
     nous throughout; (d) in two other antheridia, one very young
     and one approximately mature, the basal cell, exclusive of the
     outer membrane, was lacking in cellulose.

The distribution of cellulose would seem to be significant, as it is
conspicuously present in the resistant outer membrane of the an-
theridium, almost wholly lacking in the cap cell itself, and sometimes
conspicuous in the basal cell. The variability of its presence is also
suggestive. Probably the lack of cellulose in the cap cell is largely
responsible for the evanescent nature of its membrane.

To what extent the non-cellulose walls are pectic in composition,
and to what extent the cellulose itself exists in combination with
other substances, is not certain. However, the fact that gameto-
phytes of *Athyrium filix-foemina*, soaked in Javelle water (as sug-
gested by Dr. PAUL B. SEARS) from one to several days, gave excep-
tionally good bluing in the hydrocellulose reaction, indicates the
presence of a combined cellulose. It appears that the Javelle water
dissolves out certain wall constituents that otherwise hinder the
hydrocellulose reaction. Identification of these interfering sub-

stances has not been made. Pectic materials are known to be present; protein might well be expected in these young walls; and fatty materials offer a third possibility.

TUPPER-CAREY and PRIESTLEY (35) have studied in detail the cellulose reactions of young walls. They suggest protein complexes as responsible in certain cases for resistance to sulphuric acid, while fatty substances may inhibit the reaction with chloriodide of zinc. They used Javelle water to dissolve out cell contents, but do not suggest that it dissolves the complex protein of the walls, unless followed or preceded by ammonia, in which case it would also remove interfering fatty substances. The conclusions of TUPPER-CAREY and PRIESTLEY were further supported by an experiment involving digestion with enzymes.

Protein tests have been made with *Athyrium filix-foemina*, but without special reference to the walls. Positive reactions were obtained with Millon's reaction and the xantho-proteic reaction. The biuret reaction was very faint. The color in each case was in the spermatogenous mass. It is recognized that certain other substances could give these reactions.

Tests for fatty substances have involved primarily the tests for cutin. Although gametophytes from a number of species were tested, the results were hardly convincing and were in general negative. The walls of rhizoids and antheridia of *Athyrium filix-foemina* were resistant to a 3-day period in concentrated sulphuric acid at room temperatures. Sudan III occasionally gave a slight reddishness of the membranes, as checked with the use of compensating ocular and apochromatic objective. Saponification tests have been negative; but a separation of the wall into two layers was noted about the antheridia and marginal prothallial cells. The use of osmic acid indicated the presence of diffuse fatty material in the prothallial cells, the peripheral antheridial cells, and particularly at the apex of a young antheridium. The reagent was used as suggested by GARDINER and ITO (11) for a tannin test.

Pentosan tests were also made. The usual tests with orcin or phloroglucin were negative, or occasionally indicative of the presence of a trace of pentosan material. With Matthew's orcin, on the contrary, a dull brick red (that is, a pale color with red predominat-

ing but having also a blue quality) was obtained regularly in the spermatogenous mass of antheridia of *Onoclea sensibilis*, *Cyrtomium falcatum*, and *Aspidium simulatum*, when heated several minutes on the boiling water bath.

When gametophytes of *Athyrium filix-foemina* were hydrolyzed for an hour or more (over the boiling water bath) with 20 per cent hydrochloric acid, a reddish color developed which became most conspicuous about the sperm mother cells. The use of either orcin or phloroglucin (as in the usual pentosan tests) increased the intensity of this color, indicating that the hydrolysis liberates pentoses (possibly from the pectic materials present), and that the gametophytes and particularly the antheridia contain some substance capable of giving the color reaction with the pentoses (probably a phenol). Tests for galactan were also made. With *Cyrtomium falcatum* and *Athyrium filix-foemina*, insoluble colorless crystals were obtained which compared favorably with those similarly prepared from agar-agar, and with those of pure mucic acid. After hydrolysis with hydrochloric acid, many crystals of cuprous oxide appeared with Flückiger's reagent. There seemed to be more of this precipitate in hydrolyzed than in unhydrolyzed material, possibly indicating a breaking down of hemicelluloses into sugars. Accurate quantitative work was not done.

A general mucilage test was made, using methylene blue as suggested by YOUNGKEN (38). The thalli were mounted in the reagent for 3 minutes, lifted out and touched to filter paper, mounted in glycerin and observed. The results were as follows:

A. FIRST OBSERVATION:

 1. Spermatogenous masses very blue.
 2. Peripheral cells slightly blue.
 3. Prothallial cells not blue.
 4. Rhizoids blue.

B. LATER OBSERVATION (several hours later, after the excess blue had faded out):

 1. Spermatogenous mass a deep purple blue, darker at base of funnel.
 2. Peripheral cells a decided light blue.
 3. Rhizoids a similar light blue.

The additional tests made included those for tannins, starch, and sugars.

For starch, the iodine reagent was used. Starch granules were found in the sperm mother cells, apparently in the vesicle of the sperm cell. Their presence has been noted in the sperms of various ferns by Buller (6), Mottier (21), Allen (1), and Campbell (7). Starch was also frequently found associated with the chloroplasts of the peripheral cells of the antheridium, but sometimes was lacking. These chloroplasts are regularly smaller than those of adjacent prothallial cells. The gametophytes used included the following species: *Cyrtomium falcatum*, *Onoclea sensibilis*, *Aspidium simulatum*, and *Woodwardia orientalis*. It is interesting to note that the basal cell sometimes became browner than the other cells, in the iodin reagent.

The observation of many granules in the cytoplasm of the peripheral cells during the starch tests suggested the possibility of the presence of tannin. The osmic acid test after Gardiner and Ito (11) was first used. The results were indefinite, but in general negative. Later the tannin tests outlined by Eckerson (10) were used, and supplemented by other tests suggested by Haas and Hill (14). Most of this work was done with prothallia of *Onoclea sensibilis*. In general, the presence of a trace of tannin was indicated.

Sugars are present in the antheridia as indicated by Flückiger's reaction. The cuprous oxide crystals of the fern material appeared in clusters or balls rather than being scattered, as in tests with the pure sugars. Enough copper tartrate was used to make the drop of solution decidedly blue. Good localization was obtained about the antheridia of *Onoclea sensibilis*, after one minute's heating on the water bath. The crystals were particularly abundant about the cap cell (fig. 20). At least 73 antheridia on one slide gave a localized test. Longer heating gave scattered crystals, especially abundant among the rhizoids. With *Cyrtomium falcatum* the crystals were most conspicuous on the ring cell, but also abundant about the cap cell.

Osazone tests were also made, but definite identification of the sugars present is not possible at this time. Drawings of osazones appear in figs. 8–10, on antheridia of *Onoclea sensibilis*. Osazones were also obtained on antheridia of *Cyrtomium falcatum*.

Whether these sugars function in an osmotic mechanism that causes the swelling of the peripheral cells of the antheridium cannot be determined without further study.

General summary

1. This investigation of antheridial dehiscence in the Polypodiaceae was based on living material, both morphological and microchemical studies being made.

2. Extrusion of the intact cap cell has been found to be a characteristic method of antheridial dehiscence, as described by SCHLUMBERGER.

3. One significant modification of the extrusion of the cap cell has frequently been observed, the extrusion of cap cell contents as a granular mass.

4. In all cases there is a hole or pore in the outer membrane of the antheridium.

5. A star-shaped rupture of the apical cell, in the sense of the earlier writers, does not occur.

6. The wall cells are active in the opening; but the swelling of the spermatogenous mass seems to be primary in causing the initial tear in the outer membrane.

7. The pectin of the sperm mother cell membranes seems to cause swelling of the spermatogenous mass.

8. The wall cells are also partly pectic in nature, a fact which may account, at least partially, for their ability to swell.

9. The cap cell is markedly lacking in cellulose, a fact which may account for the observed evanescent nature of its inner membrane.

10. The presence of sugars in the peripheral cells is indicated.

11. *Athyrium filix-foemina* should be added to the list of polypod species in which the cap cell of the antheridium sometimes becomes divided.

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DEVELOPMENT AND VASCULAR ORGANIZATION OF THE FOLIAR ORGANS OF CARYA CORDIFORMIS

LaDema M. Langdon

(WITH PLATES I, II AND FOUR FIGURES)

Introduction

This paper, continuing an investigation in seedling anatomy first reported in 1927 (**12**), presents the results of a somewhat detailed study of the development of foliar organs in *Carya cordiformis*, with particular emphasis on the origin and differentiation of the vascular tissues of the leaf and scale primordia of seedling buds.

A great number of papers have been published relating to the subject of foliar morphology since the studies of DE CANDOLLE (**1**), NAGELI, and TRECUL; but in these earlier accounts, references to the origin and longitudinal differentiation of the vascular tissues of the leaf, or exact relation of the foliar strands to the organization of the primary vascular axis, have been indefinite and often contradictory. Concerning the position of meristem in the leaf, NAGELI (**13**) early established from his examination of certain of the phanerogams (*Utricularia, Astragalus,* and *Myriophyllum*) that the leaf possesses originally an apical vegetative point; but this may be the first to pass over into permanent tissue, while at the base of the leaf cell formation takes place freely, inasmuch as the tissue there retains its embryonal character.

TRECUL (**15**) found that development of lateral members of a leaf may proceed sometimes acropetally, sometimes basipetally, or from the middle both upward and downward; further, that this process of leaf formation may show variance in different although closely allied plants. His error in considering that the leaf sheath was the first to arise was later corrected by EICHLER (**5**), who found that the base of the leaf does not at once take on the character of the leaf sheath, but the sheath is differentiated later only by intercalary growth out of the basal portion of the leaf.

With regard to the laying down of the leaf surface, PRANTL (14) distinguished three types of leaves: (1) Those of the basiplastic type, where the stretching takes place at the apex of the primarily uniform embryonal primordium, and proceeds downward until nearly the whole of the active meristem disappears. This is found in the Musci, *Lycopodium*, Coniferae, most monocotyledons, and a number of dicotyledons. Where, as in dicotyledons, feathered veins occur, a strong midrib is first of all differentiated, and this is accompanied both right and left by meristic tissue, which passes over into stretching tissue successively in a basipetal direction, and at the same time in a transverse direction. (2) In the pleuroplastic type, where the meristem is marginal, the leaf apex does not pass into the permanent condition as rapidly as in the basiplastic type. The transition into stretching tissue takes place in the whole tissue arising out of the meristem at nearly the same moment. When branchings occur they proceed in acropetal succession, as in *Quercus*, *Corylus*, and *Tilia*. (3) In the eucladous type the branchings do not proceed, as in the two former types, only when a portion of the meristem has commenced to stretch, but appear when the leaf is still one uniform mass of embryonal tissue. This is seen in *Ginkgo*, *Juglans*, and the Papilionaceae.

Of the later publication dealing with the ontogeny of foliar organs of the dicotyledons, those of FOSTER (6) are the most recent and most detailed. In his studies of the periodicity and comparative history of development of the foliage leaves and cataphyllary structures of the horse-chestnut, he concludes that the bud scales and the lower transitional forms in *Aesculus* are two-membered foliar organs in which the variously developed "lamina" is homologous with the blade of the foliage leaf; while the characteristic and polymorphic sheath is essentially a winged structure, and represents a completely divergent product of the "hypopodium" (leaf base). GOEBEL's theory of "arrested formations" he finds inapplicable to the situation in the horse-chestnut, and concludes that the cataphyllary structures develop from primordia in which there is an almost complete reversal of the growth rates existing between the lamina and the "hypopodium" in the foliage leaf initial.

The problem of distribution and order of appearance of the veins

(primary and secondary) of the leaves of monocotyledons and dicotyledons has been dealt with in some detail by most of the writers mentioned here and others, but in practically all accounts a vague but commonly accepted theory is that leaf traces, vascular strands connecting the vascular supply of the blade with that of the stem axis, are "put forth" at different points from the vascular cylinder, and differentiate toward the leaf. In their progress through the sheathing leaf base they meet and anastomose, or branch freely, eventually becoming continuous with the primary vascular supply of the petiole and lamina.

TRECUL (16), in his study of venation in the leaves of *Aesculus*, describes the primary bundle of the scales as ascending from the stem and progressing toward the median leaflet. Other primary bundles arise a short distance to either side, successively beneath the first and second lateral leaflets, and elongation of these bundles in the leaflets is from base to apex. Secondary bundles arise later, interposed between the primary bundles (later connected with them), and in their passage toward the tip of the scales they fork, supplying branches to adjacent ribs of neighboring leaflets.

The venation typical of monocotyledons arises according to GOEBEL (7, 8) "when a primordium of a leaf attached by a broad base to the stem, grows nearly uniformly in length and breadth (but at different times)." The conducting bundles which "enter" the leaf, and out of which the median is formed, traverse its whole length nearly uniformally from base to apex. Among the dicotyledons two cases cited are: (1) that of *Acer platanoides* (a palmate leaf with a divergent type of primary veins), where the middle nerve enters the leaf first, then in order the veins of the two upper and two lower leaf lobes; (2) that of *Fraxinus excelsior* (a pinnate leaf with acropetal succession of pinnules), in the broad base of the leaf primordium of which a large number of conducting bundles radiate from one another in correspondence with the growth of the pinnules. No precise information is given in either case as to the point of origin or direction of differentiation of the primary laminar bundles.

According to EAMES and MACDANIELS (4), the term leaf trace may be applied to any bundle which extends to a leaf, or to the complex of bundles supplying a given leaf. In *Quercus* and *Carya*, I have

found that origin and longitudinal differentiation of the procambial or desmogen elements composing the leaf trace commence in the leaf base, and progress from that point basipetally until they meet and merge in the primary vascular axis with like elements of other traces. This seems to be in agreement with HABERLANDT's (9) observations regarding the "dicotyledonous type" of vascular structure.

Investigation

Materials and methods

For the purposes of the major part of this study, seedlings of *Carya cordiformis* 3–8 weeks old, with plumules ranging from 2.5 mm. to 6 cm. in length, were selected; and, as in the case of *Quercus* (12), serial paraffin sections were secured through the plumules from tip of main axis to the cotyledonary plate. Dissections of seedling buds were to some extent used to supplement the serial preparations, successive scale and foliage leaves being removed with the aid of needles, cleared in alcohols and xylene, and mounted in damar. When examined with a binocular microscope (16 mm. obj. and 5 oc. combination) under strong artificial light, considerable internal as well as external detail is distinguishable in these cleared preparations.

Fixation of the sectioned material was with formalin-acetic-alcohol (2, 11) or with Flemming's fluid (weaker solution). Sections were cut 8–10 μ, and staining in practically all cases was with safranin-light green or with Flemming's triple stain, the latter proving most satisfactory in the differentiating of meristematic tissues.

The foliar organs, formed by the primary axis of seedlings of the stages just described, consist of 6–8 scale leaves, and 5–6 foliage leaves and primordia. The transition from scale to foliage leaf may be regarded as somewhat gradual, for the first-formed foliage leaf is reduced to one leaflet (with at times the rudiments of two others), while the scale leaves immediately preceding the first foliage leaf display a marked, although reduced, laminate structure.

The scale and foliage leaves from their early stages of development are more or less completely enveloped in a dense tangle of hairs: unbranched unicellular, multicellular protective, and glandular. The latter, illustrated in different figures of this paper, are of several types. Some are unicellular or small multicellular, short-

stalked, capitate glands (figs. 7, 8, 10, 12), their secreting cells thin-walled, rich in protoplasm, and but slightly vacuolate, very likely early stages in the development of larger glands; others are mucilage or resin-secreting glands of an elaborate type (figs. 8, 9, 11, 14, 21), consisting of a base and a one-two-celled stalk surmounted by a multicellular cup- or disk-shaped gland. In such mucilage or resin-secreting glands, according to VON HANSTEIN (10), the secretion is generally a constituent of the cell membrane, appearing in a constantly increasing quantity between the cuticle and the cellulose layers of the wall, until the cuticle is distended in a vesicular manner and finally ruptured. Whether the raw materials from which the secretions are made are derived directly from the cell contents, however, or entirely from metamorphosed layers of the cell membrane, is a question as yet undetermined.

Both the glandular and the protective hairs may offer some impediment to sectioning, particularly the protective hairs on the older scale and foliage leaves, owing to their thickened walls and the accumulation within their cells of resins and tannins. The same difficulty is encountered in the preparation of oak buds; here, however, the problem is further complicated by the unyielding and brittle protective stipules.

MERISTEM AND PRIMARY TISSUES OF AXIS

Cases of terminal growth by means of several apical cells, such as characterize the leaf stems of the majority of angiosperms, have been considered in some detail by several investigators, including HANSTEIN (10), DOULIOT (3), and HABERLANDT (9). HANSTEIN distinguished in the stem tip of certain of the dicotyledons three distinct strata or "histogens," giving rise to the plerome, periblem, and dermatogen. These histogens are reported to appear at the first divisions of the embryo, maintaining their independence throughout the life of the plant, and are increased each by its own group of initials. DOULIOT, whose work on terminal growth (3) has already been reviewed (12), appears to have placed more emphasis on a "vertical" series of generative cells in dicotyledons, 2–3 initials superimposed from which epidermis, cortex, and central cylinder are derived.

HABERLANDT has summarized the three possible ways in which

several initials may be associated as follows: (1) two to several apical cells all located in the same horizontal plane, juxtaposed or horizontally seriated; (2) apical cells (2 or 3 in number) situated in successive horizontal planes, superimposed or vertically seriated; (3) cases where the apical cells are arranged in successive strata (that is, both juxtaposed and superimposed). This third type is by far the most difficult of observation, but probably widely distributed among angiosperms.

SEEDLINGS.—With the appearance of the first foliar organs, the terminal apices of *Carya* seedlings are so complicated by rapidly forming primordia that it becomes difficult to distinguish the vegetative point and initials of the stem from the generative cells of the youngest leaf primordia. In certain of the younger axillary shoots of the seedling, however, as well as in the primary axis of winter buds of this genus, it has been possible to distinguish a stem tip. In fig. 25, a median longitudinal section through a young axillary shoot of a seedling of *Carya cordiformis*, there appear two successive horizontal groups of initials and a single basal initial, the 3 units associated at the apex of the stem's axis in the form of an inverted cone or wedge. The initial cells of the upper series, 3 or 4 in number, from which the dermatogen is derived, all meet at the center or adjoin the median plane of the axis. Immediately beneath these can be seen a second series of 4 initials from which by periclinal divisions a cortical section of the stem is derived; and at the apex of the wedge a single large 6-sided initial, with 5 cutting faces, one basal and 4 lateral, from which the central portion of the stem's promeristem takes its origin. A stratification of the meristem due to this vertical seriation of the initials is evident for only a short distance behind the apex. It soon becomes difficult to determine just how much of the promeristem may be traced to the second series and how much to the single basal initial. Fig. 26, surface view of a portion of the stem tip and 3 primordia of the primary axis of a winter bud, demonstrates the arrangement of the upper series of initials of the stem axis, as also the relation of segments cut from these initials.

Figs. 3, 4, 7, 10, and 11 are representative of conditions found at different levels in the primary axis of *Carya* seedlings. Fig. 3, a transverse section just above the tip of the axis, shows in outline the

petioles of four leaves and the bases of the primordia of a fifth and
sixth foliar appendage. Fig. 7 shows in detail a section of the primary
axis at the level of the stem apex and slightly above the point where
the youngest primordium (L_6) merges with the stem tissues. In this
region there appear, distinctly outlined, six small masses of pro-
cambial tissue, five of which are associated with the base of the fifth
or second youngest primordium, while strand ms marks the position
of the central or first procambial strand of the sixth or youngest
primordium. The three median primary strands of the fifth leaf are
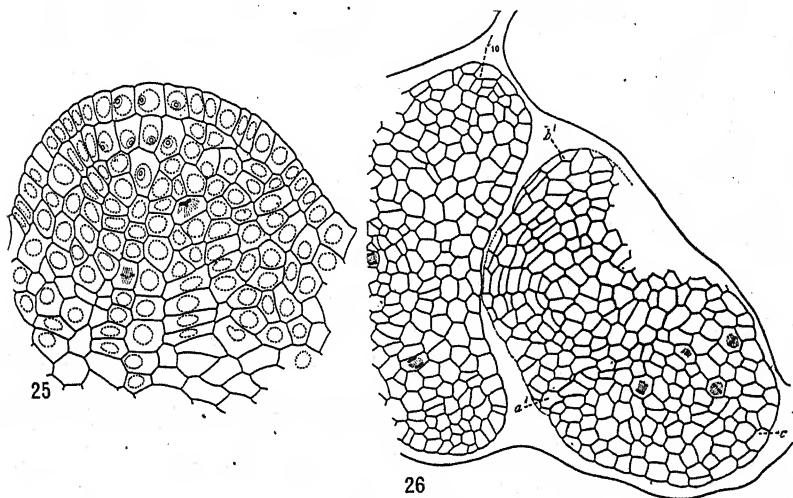
FIGS. 25, 26.—*Carya cordiformis:* fig. 25, median longitudinal section through young
axillary shoot of seedling, showing promeristematic tissue and vegetative point of stem;
×533; fig. 26, surface of apex of primary axis of winter bud, showing portion of stem
tip and three primordia (a', b', c'), also section base of tenth leaf; ×229.

seen drawing toward the center; also the left and right lateral pri-
mary strands of the same primordium.

At the second node from the apex (fig. 10), a point 48 μ below that
illustrated in fig. 7, there is a well organized primary vascular cyl-
inder, interrupted only by the promeristematic tissue of a secondary
shoot borne in the axil of the fourth leaf. All elements of this pri-
mary cylinder, as yet in the procambial condition, have their origin
at the bases of primordia enveloping the axis; and in the course of

their basipetal differentiation they enter the primary vascular cylinder, becoming its "common" bundles.

The foliar origin of the procambial elements of the primary vascular axis is also apparent in fig. 11, detailing the basal portion of the fourth leaf at its point of union with the stem. The primary laminar strands (lateral and median) mark the position of the 4 or 5 outward projecting points or ridges of the primary cylinder, while the desmogen strands (described as taking their origin concurrent with that of the primary traces, but in the extreme lower section of the primordial leaf base and differentiating chiefly basipetally) occupy the slightly depressed regions of the cylinder. The latter (*a*, figs. 10, 11), although clearly defined at the second and third nodes from the apex, are still undifferentiated procambium. At the fourth and fifth nodes these connecting strips of primary vascular tissue can be distinguished as discrete bundles occupying the areas between the median and lateral primary traces, but maturing later than the primary laminar strands (figs. 5, 24).

FOLIAGE LEAF

The foliar organs of *Carya cordiformis*, both foliage and scale leaves, are arranged alternately in 5 vertical ranks on the stem axis, with the sixth over the first, the seventh over the second, the eighth over the third, etc., the usual 2/5 leaf arrangement so common in alternate-leaved dicotyledonous plants.

Each adult foliage leaf of the seedling consists of a leaf base (conspicuously sheathing), a petiole, and a lamina of 1–5 broadly lanceolate, slightly stalked leaflets, pinnately arranged. The number of leaflets forming the lamina of the adult foliage leaf in winter buds ranges from 7 to 9 or even 11. It is evident, therefore, that considerable variability in leaflet number occurs both in seedling and winter buds of *C. cordiformis*.

In seedlings 6–8 weeks old, the lamina of the first-formed foliage leaf frequently consists of but a single leaflet. The second, third, and fourth in order of appearance are usually tripinnate (figs. 1–3), while the fifth bears the primordial lobes of five leaflets, and the sixth and seventh are each unilobate. In younger seedlings (3–4 weeks old) the lamina of the first foliage leaf is also unipinnate, the

second may be bi- or tripinnate, the third tripinnate, the fourth displays 3–5 leaflet primordia, while the fifth and sixth are each unilobate. In the foliage leaves of winter buds there is an increase in number of leaflets (7–9) with successive leaves, that is, to the sixth leaf; but in later-formed leaf primordia, a decrease from seven to five or even three leaflet primordia. The last two sets of primordia borne at the stem tip are apparently primordia of scale leaves.

FOSTER (6) has pointed out that the number of leaflets in the adult foliage leaf of the horse-chestnut decreases with each successive pair of leaves, while in the cataphyllary series there is noted a tendency for the number of leaflet primordia to increase progressively from five in the lower scales to seven in the upper cataphylls. The causal factors responsible for this fluctuation in leaflet number, as well as the asymmetrical formation of leaflet primordia, he believes are completely obscure.

DEVELOPMENT OF PRIMORDIUM.—No exact determination of the number and arrangement of the generation cells of the leaf of *Carya* has as yet been made, although the preparation of embryonic stages essential for such a determination is in progress.

The foliage leaf primordium makes its appearance as a small, knoblike emergence, which by rapid meristematic growth soon extends as a ridgelike papilla on either side of the slender vegetative point to about one-half the circumference of the axis. The bases of the two youngest primordia not only meet but in some cases actually merge above the growing point. After the formation of the first scale leaves, so closely do these primordia succeed one another that it becomes practically impossible to distinguish the vegetative point or promeristematic region of the stem from the youngest primordium of a leaf. In vegetative points with such close-set primordia of leaves, GOEBEL (8) observed that not infrequently there is no free surface of the growing point left, and in such cases the lower portion of the leaf primordium remains frequently united with the surface of the shoot. There follows a period of general and rapid meristematic growth in the primordial leaf, in the course of which the lobes of the first primary segments of the lamina appear, the median one first and well in advance of the first laterals. It is the writer's observation that subsequently formed lateral leaflet primordia in the foliage or-

gans arise when the central portion of the embryo leaf has com-
menced to stretch, and appear as successively developed lateral
lobes on the marginal meristems of the "upper leaf." With the for-
mation of the first two lateral leaflet primordia, primary segmenta-
tion or development of leaf members of the first order may cease, as
is the case in the scale and first three or four foliage leaves of the
seedling.

If other leaflet primordia are formed, they appear in acropetal
succession, the primordium of the third leaflet above the first lateral,
the fourth above the second lateral, etc. The leaf primordium at this
point (fig. 18 P_6) consists of a diminutive base, and a 3–5-lobed
lamina with no evident elongation of the zone between the leaf base
and the blade. Stretching commences at the apex of each embryonic
leaflet, and proceeds downward with early differentiation into mid-
rib and marginal meristems (fig. 19). The median or first-formed
leaflet may have progressed to this stage of differentiation while the
laterals are still blunt, uniformly embryonic projections. Marginal
teeth take their origin at a later period as lateral projections from
the marginal meristems of each leaflet.

Differentiation of the desmogen or procambial elements of the
primary vascular bundles takes place early, and is first evident in
the primordial leaf base. As stated previously (12), the elements of
these strands may be distinguished from the surrounding tissues by
the size and shape of the cells and the character of mitotic activity.
In an embryonic leaf consisting of three leaflet primordia, differentia-
tion of procambium occurs at three points in that region of the pri-
mordium destined to become the upper section of the leaf base. The
first strand appears while the primordium is still in the unilobate
condition, as a group of desmogen elements occupying a central posi-
tion beneath the median leaflet lobe. Other primary strands arise
later, with the evolution of lateral pinnae, one beneath each of the
primary lateral leaflet primordia.

With continued meristematic activity in the basal section of each
leaflet primordium, "secondary" desmogen strands (varying in num-
ber) arise to either side of the differentiating primary strands. The
progression in development of these several groups of strands (both
primary and secondary) is from their point of origin both acropetal

and basipetal. Acropetally they enter the five primordial lobes of the leaf, and their further development in this direction keeps pace with the growth of the leaflets, the median "primary" strand of each group being the center bundle of the primary midrib of each leaflet. Basipetally the differentiation of the desmogen strands progresses by repeated longitudinal division of the intervening elements of the leaf base and cortex of axis. In their basal differentiation there occur in the upper section of the leaf base an approximation and anastomosis of the primary and secondary strands of each leaflet, so that
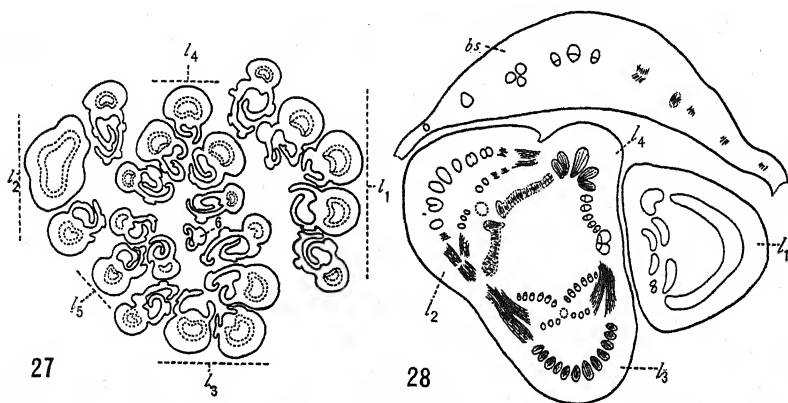
FIGS. 27, 28.—*Carya ovata*: fig. 27, transverse section of upper portion of winter bud; pinnae of six of oldest foliage leaves illustrated; ×17.8; fig. 28, transverse section through basal portion of same bud, showing vascular organization of basal sections of second and third leaves, and connection of vascular strands of fourth leaf with primary axis; ×29.8.

the several bundles ordinarily found at the base of the petiole are reduced to a median group of 3–5 bundles, and two lateral groups each consisting of 1–3 strands; and thus, as three separate groups, they approach and connect with the primary vascular axis (figs. 4, 5, 10, 11, 28).

While differentiation of the procambial elements of the "primary" bundles of the leaflet primordia is in progress, there may be distinguished in the broadening leaf base the desmogen of yet another set of vascular strands. These strands (5–9 in number) take their origin at a lower level in the leaf base than strands supplying the

lamina of the leaf, and differentiate acropetally only to a point of
union with the median and lateral laminar bundles. Basipetally they
maintain a direct and independent course (*a*, figs. 10, 11, 24), enter-
ing the primary vascular axis to either side of the median laminar
strands. Such bundles occupy that portion of the leaf base extending
between the median and lateral primary laminar groups, and upon
entering the primary cylinder occupy the slightly depressed regions
of the vascular axis, while the laminar strands (lateral and median)
mark the position of the four or five outward projecting points of
that axis. These strands were not definitely determined in the earlier
study (12) of *Quercus* seedlings, but more recent examination has
demonstrated their occurrence both in *Quercus* and in *Carya*. It is
thus apparent that the number of vascular bundles entering the
stem at each node is much greater than commonly believed; the
number varying with different foliar appendages in *Carya*, averages
17–18 for the second foliage leaf (fig. 5), 16 for the third, and 10–12
for the fourth and fifth (figs. 10, 11), with an appreciably smaller
number of traces contributed by the scale leaves.

In *Carya*, as in *Quercus*, stem bundles may be distinguished in
longitudinal sections of the epicotyl early in its development, in
stages preceding leaf formation. But with the appearance of foliar
appendages, so close is the succession of leaf primordia at the apex
of the stem, and so reduced the extent of the apical meristematic
region of the stem axis, that cauline strands no longer are distin-
guishable, and it is altogether probable that the apical meristem be-
comes from this time wholly occupied with the formation of pri-
mordia. Thus with the formation of leaves or leaflike appendages
at the stem tip, all vascular strands of the primary vascular cylinder
are "common" bundles, which enter the stem from the leaf base
along a curved path, and afterwards differentiate longitudinally
through two or three short internodes, sooner or later connecting
laterally with procambial strands from earlier formed leaves.

Continued development within each leaflet involves further differ-
entiation of the primary midrib, and marginal meristems, with the
origin within the marginal meristems of other centers of lignification
which differentiate basipetally to meet the primary and secondary
laminar strands. Later intercalary meristematic growth in the pri-

mordial leaf leads to formation of a stalk between the blade and the leaf base.

Lignification of all vascular strands commences at the point of origin, in the case of the primary foliar strands with the procambial cells of the leaf base, and progresses with the advance of these elements inward toward the stem, and outward and upward toward the tip of the leaflets.

SCALE LEAVES (BUD SCALES)

The primary axis of seedlings, 3–8 weeks old, usually bears 6–8 scale leaves. These appendages, excepting those immediately preceding the first foliage leaves, are small, reddish brown, leathery bodies with a broad base which narrows gradually upward, and bears at the tip a rudimentary leaf lamina consisting of a median pointlet and two feebly developed lateral segments (figs. 15, 16). The sizes of such scales may with successive members show considerable variance; that of the oldest or first formed averaging 0.9–1.1 mm. in length, the second and third pairs 1.2–1.4 mm., while scale leaves immediately preceding the first foliage leaves often attain a length of 1.7–2.5 mm. The length of the first foliage leaf (possibly a transition type) may not exceed 3–4 mm. (inclusive of lamina, petiole, and base). Further, with successive bud scales there may be observed an increase in the size and prominence of the trilobed leaf lamina, that is, through the first four or six scales; the last formed scale leaf consists of a leaf base 0.5–0.6 mm. in width in adult condition, narrowing gradually to an elongated sharply pointed single-lobed lamina.

The vascular supply of each of the first two or three pairs of scales usually consists of three small laminar bundles, which in their basipetal development through the base of the appendage unite, and as a single strand connect with the primary cylinder (fig. 23). The later formed scale leaves may exhibit a more extensive vascular system, some 7–9 strands (fig. 17), which in their basipetal progression traverse the thickened median central region of the sheath of the appendage to that point in the base where the usual anastomosis occurs, reducing the 7–9 bundles to five. These five strands connect with the primary vascular axis at three points, three of the five form-

ing the median leaf trace, the fourth and fifth, as lateral strands, describing partial arcs through the leaf base to the lateral leaf gaps (fig. 22).

The primordia of the scale leaves appear in close succession at the growing point, and, as in the case of the foliage leaf primordium, each is first distinguishable as a small knoblike structure which by rapid meristematic growth soon extends as a ridge over and about the vegetative point. For a short time (varying in different scales) development of the laminar portion of the primordium parallels that of the foliage leaf. Shortly after the formation of the first 3 primary segments, dominant growth commences in the sheathing portion of the appendage, preventing further elaboration of the lamina.

The procambial elements of the primary laminar bundles are differentiated early in the embryonic base of the scale, one strand at the base of each of the three lobes of the laminar primordium. Longitudinal development of these strands is from their point of origin both acropetal and basipetal. The procambial elements identified with the lower section of the base of the foliage leaves are also found in the scale leaves, appearing concurrently with the origin of the median primary bundle of the blade and to either side of that bundle. Their longitudinal differentiation is chiefly in a basipetal direction.

Summary

1. The promeristem of the stem axes of seedlings of *Carya cordiformis* has its origin in three successive horizontal initial units, associated at the apex of the stem's axis in the form of an inverted cone or wedge.

2. Foliar organs formed by the main axis of seedlings 3–8 weeks old consist of 6–8 scale leaves, 5–6 foliage leaves, and primordia. The transition from scale to foliage appendage is from scale leaves exhibiting a marked, although reduced, laminate structure to uni-, tri-, and five-pinnate foliage leaves.

3. Variability in leaflet number, as well as an asymmetrical formation of leaflet primordia, occurs in the foliage leaves both of seedling and winter buds; in seedlings an increase from one to five leaflets with successive leaves, in winter buds an increase from seven to nine

leaflets in the first six leaves, but in later formed leaf primordia a decrease from seven to five leaflet primordia.

4. Lateral leaflet primordia in the foliar organs arise in acropetal succession, as successively developed lobes on the marginal meristems of the "upper leaf" of the primordium, with a reduced development of this portion of the primordium in the scale and first formed foliage leaves of seedlings.

5. Differentiation of the desmogen or procambial elements of the "primary" vascular bundles of the foliage leaf takes place early, and is first evident in that region of the primordium destined to become the upper section of the leaf base. The first strand appears while the primordium is in the unilobate condition, as a group of desmogen elements occupying a central position at the base of the median leaflet lobe. Other primary strands arise later, with the evolution of lateral pinnae, one beneath each of the lateral leaflet primordia. With continued meristematic activity in the basal section of each leaflet, primordium "secondary" desmogen strands arise to either side of the differentiating primary strands. Longitudinal development of these several groups of strands (both primary and secondary) is from their point of origin both acropetal and basipetal.

6. Concurrent with the origin of the primary bundles of the lamina is the differentiation of a set of vascular strands deep within the primordial leaf base. These strands (5–9 in number) differentiate acropetally only to a point of union with the primary laminar bundles. Basipetally they maintain a direct and independent course, entering the primary vascular axis to either side of the median laminar strands. Within the primary cylinder they occupy the slightly depressed regions of the vascular axis, while the laminar strands (lateral and median) mark the position of the outward protecting points or ridges of that axis. Such vascular elements, heretofore confused with and described as "cauline" bundles, account for the closely reticulate character of the primary vascular cylinder of woody forms like *Carya* and *Quercus*.

7. Cauline strands are distinguishable as units of the primary vascular axis of the epicotyl only through that embryonic period which precedes leaf formation.

Grateful acknowledgment is made for helpful criticism given by Professor W. J. G. Land during the progress of this study.

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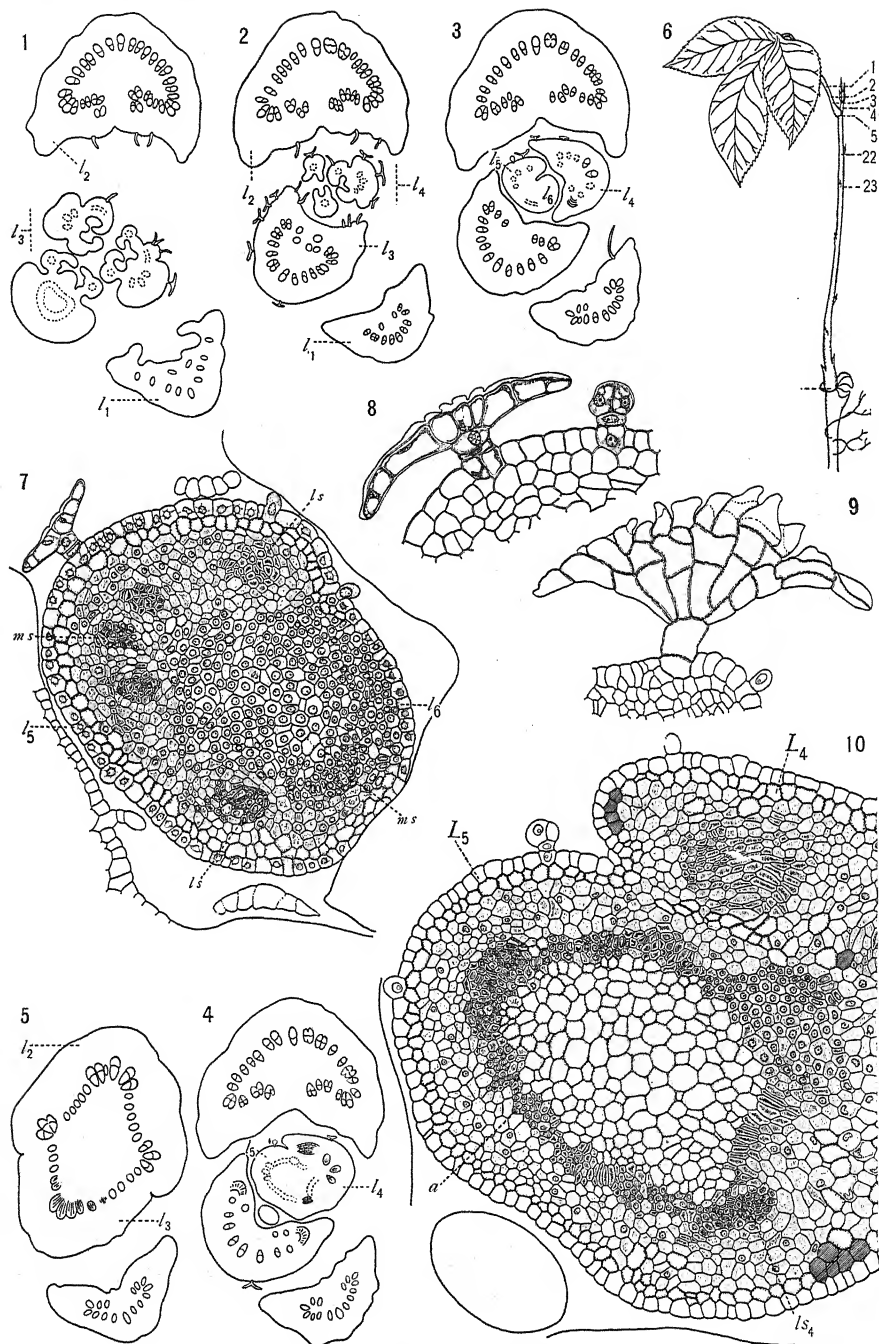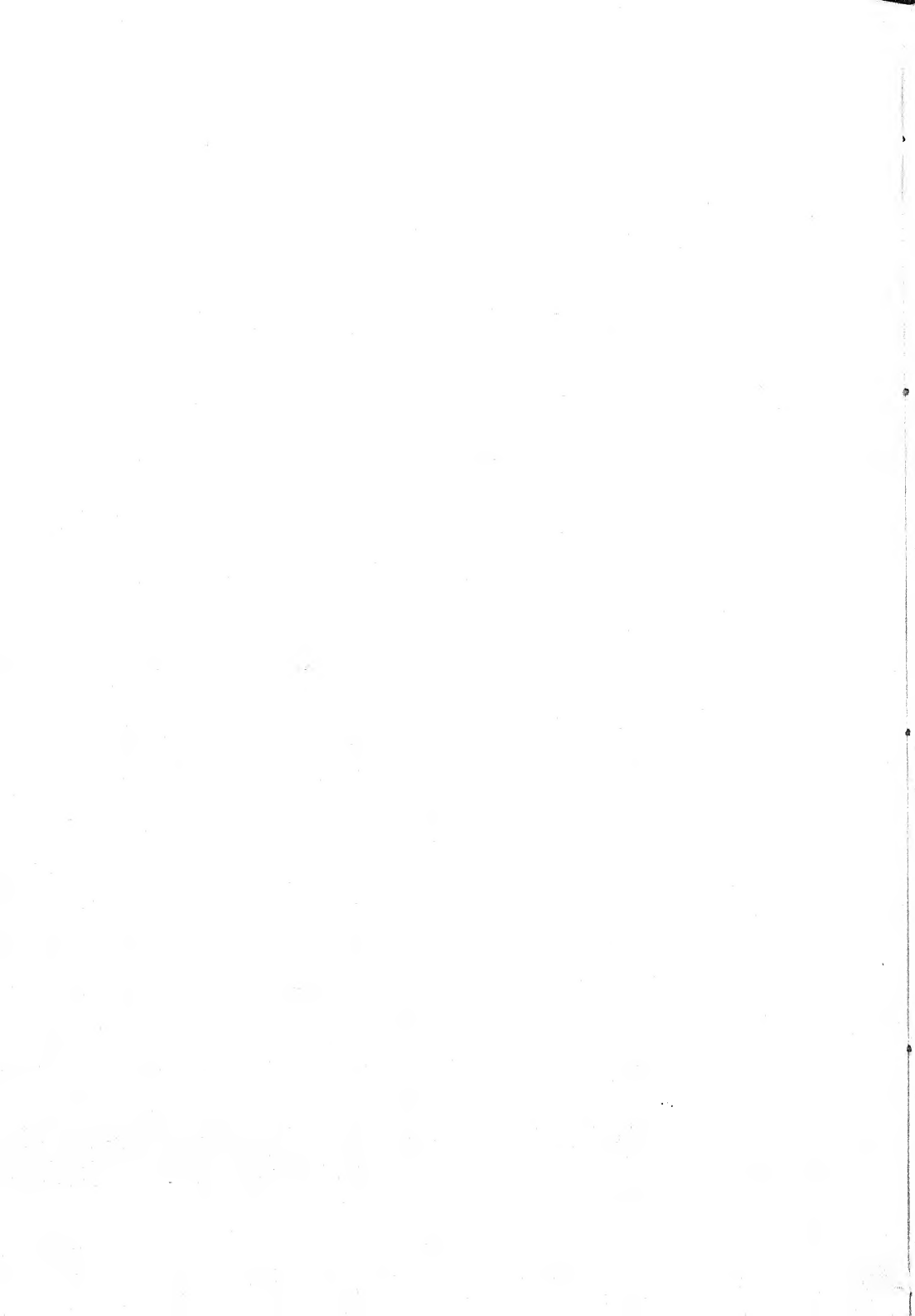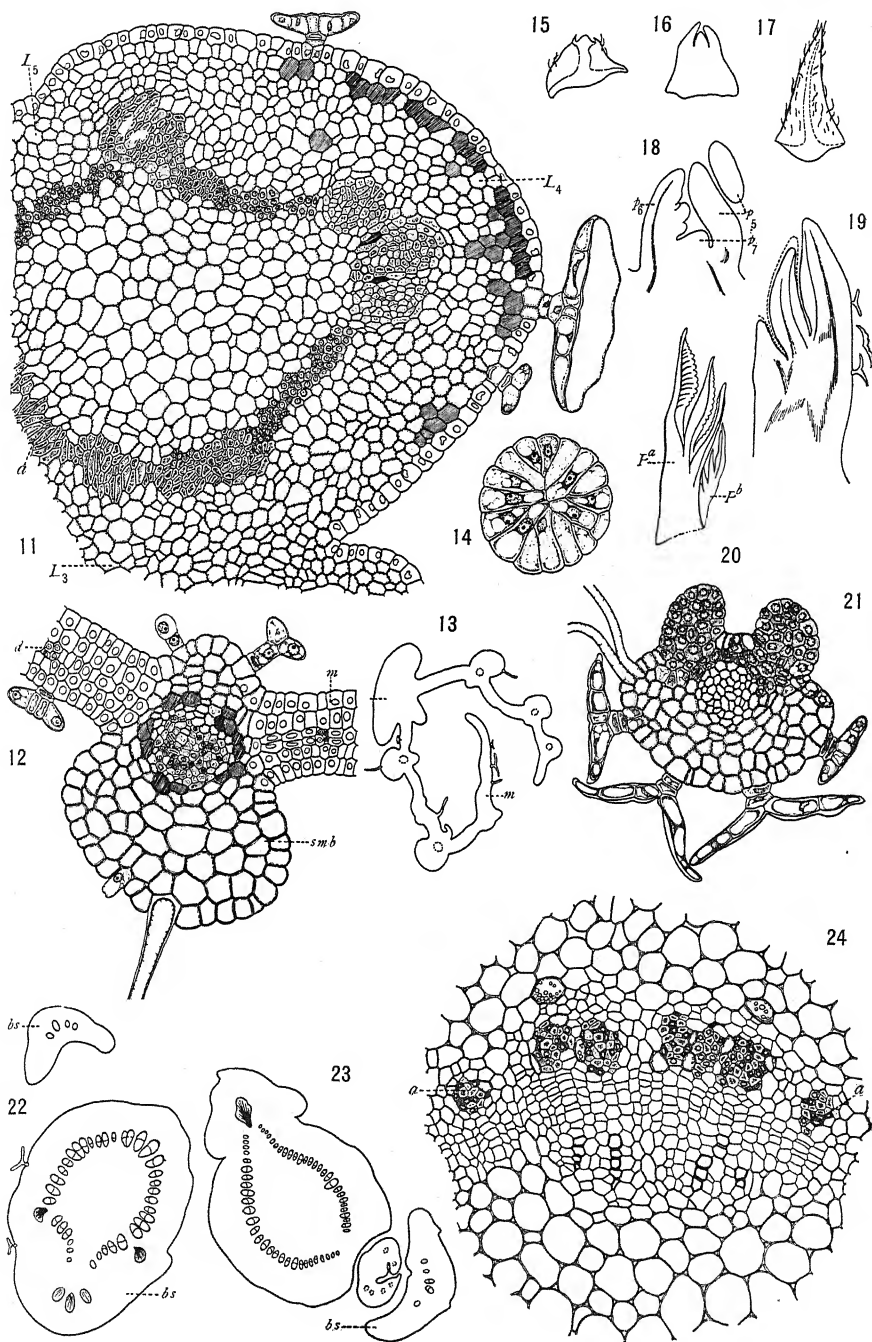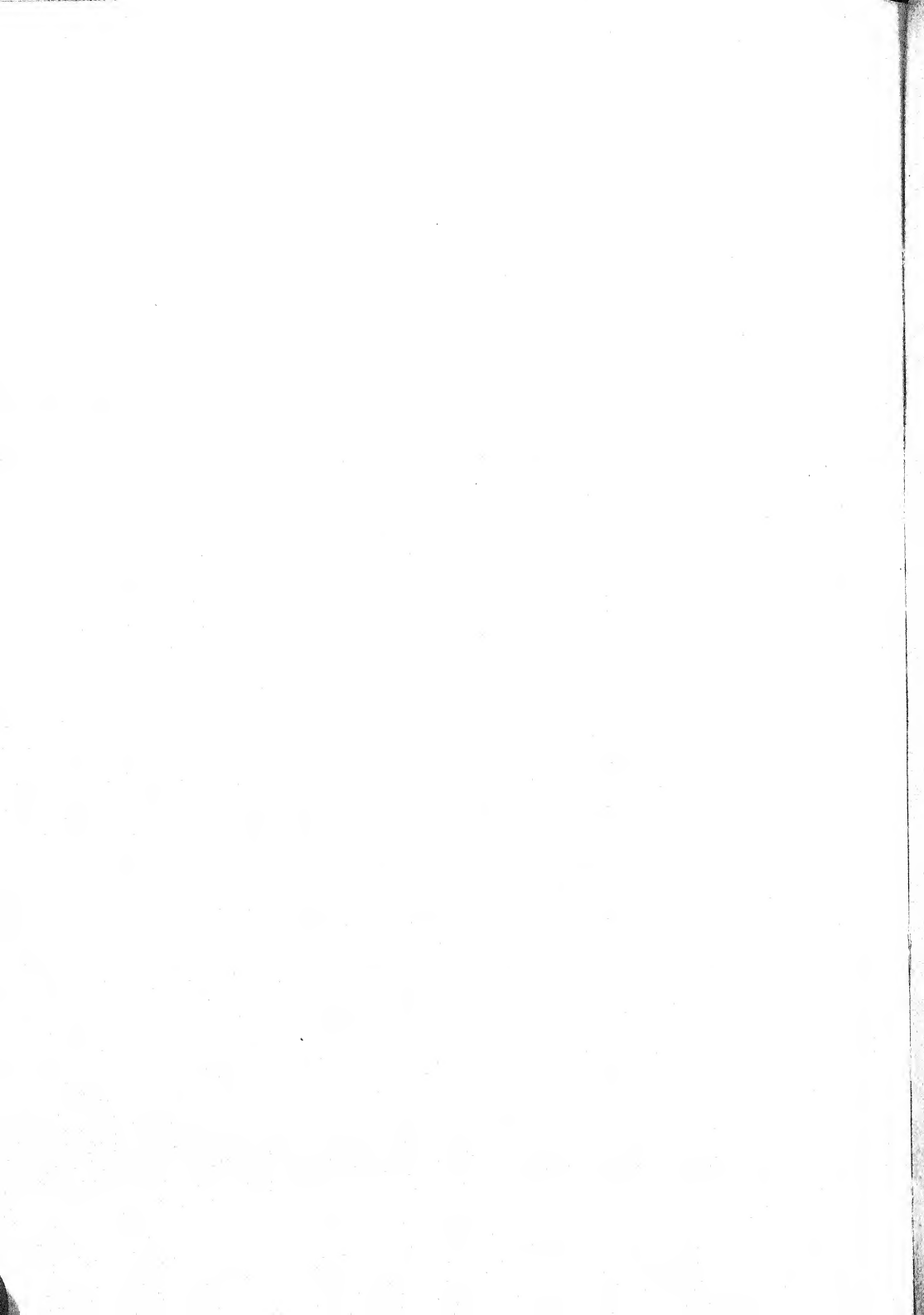
EXPLANATION OF PLATES I, II

With the exception of figs. 27 and 28, all illustrations are of *Carya cordiformis* seedlings.

PLATE I

FIGS. 1–5.—Sections at successive levels through terminal bud of 5-weeks-old seedling. L_1–L_6, first to sixth leaves in order of development; ×27.

FIG. 6.—Eight-weeks-old seedling, lettering and figures indicate approximate positions in bud of figs. 1–5; ×0.6.

FIG. 7.—Detail of terminal portion of bud 24 μ below that of fig. 3; three median "primary" strands of fifth leaf seen drawing toward center; left and right lateral primary strands of same primordium likewise differentiating toward stem axis; first formed primary strand (*ms*) of sixth or youngest foliar primordium definitely outlined; ×226.

FIG. 8.—Detail of glandular hair from ventral surface of leaflet primordium; ×320.

FIG. 9.—Glandular hair from ventral surface of mature leaflet; ×267.

FIG. 10.—Detail of stem axis and basal part of fifth primordium, 48μ below section illustrated in fig. 7; desmogen between median and lateral primary strands of second youngest primordium (L_5) clearly defined but as yet unorganized; these strands with median and lateral laminar bundles of fifth and sixth primordia now part of primary vascular cylinder, with median and lateral foliar strands occupying the points of that cylinder, the later differentiated basal desmogen the depressions; ×228.

PLATE II

FIG. 11.—Detail of basal portion of fourth leaf at point of union with stem axis; desmogen of fifth leaf to upper left side; centermost of three median strands of fourth leaf shows differentiation into primary xylem and phloem regions; left lateral of fifth primordium connects with primary cylinder; median strand of sixth primordium inserted on lower lateral of fourth leaf; ×226.

FIG. 12.—Detail of one of secondary ribs (*smb*) with adjacent blade meristems (*m*), same as outlined in fig. 13 (note prominent protective hairs as outgrowths from dorsal surface of leaflet); colleters either on dorsal or ventral face of pinna; ×267.

FIG. 13.—Transverse section of nearly mature leaflet showing primary midrib and conduplicate blade with secondary ribs; ×32.

FIG. 14.—Dorsal view of one of disk-shaped mucilage secreting glands; ×534.

FIGS. 15–17.—Successively formed scale leaves, showing increase in size and prominence of trilobed lamina with successive scales; ×9.

FIG. 18.—Median long section through apex of primary axis of an 8-weeks-old seedling, showing primordia of three foliar organs; ×53.

FIG. 19.—Longitudinal section through young foliage leaf, with lateral and median leaflets showing differentiation into midrib and marginal meristems; as

yet no indication of origin of marginal teeth from lateral meristems of each leaflet; ×53.

FIG. 20.—Foliage leaf primordia (P^A and P^B) dissected from seedling bud; ×9.

FIG. 21.—Detail of tip portion of leaflet showing differentiating primary midrib (pmb) with dorsal colleters, also ventral ridges of meristem (m) from which blade is formed; ×267.

FIG. 22.—Transverse section of seedling stem showing connection of one of later formed scale leaves with stem axis; section through upper part of node; ×32.

FIG. 23.—Transverse section of stem showing vascular connection of early formed scale leaves with stem axis; ×32.

FIG. 24.—Detail of group of foliar bundles at point of union with primary vascular axis; ×267.

COMPARATIVE STUDY OF RIVER BLUFF SUCCESSION ON THE IOWA AND NEBRASKA SIDES OF THE MISSOURI RIVER

DAVID F. COSTELLO

(WITH SEVEN FIGURES)

Introduction

A contrast in appearance of river bluffs on opposite sides of the Missouri River near Nebraska City, Nebraska, was the occasion for a comparative study of plant succession on these east- and west-facing slopes. When viewed from a distance the impression is given that trees are almost entirely absent from the west-facing Iowa bluffs. They are characterized by a very rugged topography, much dissected by erosion, and a bareness that comes of the clay soil of which they are composed (fig. 1). The east-facing Nebraska bluffs appear to be more regular in outline, with fewer dissections, and practically no promontories, and to be well covered with forest vegetation (fig. 2).

In this preliminary study, observations were confined to an area about 15 miles square. On the Iowa side, studies were made on the bluffs for a distance of approximately 15 miles north of Hamburg, Iowa, which is near the Iowa-Missouri state boundary. On the Nebraska side, observations were made on all the bluffs for a distance of 5 miles north of Nebraska City and to a point 10 miles south of that city. Thus the areas observed on different sides of the river were almost directly opposite each other. The width of the flood-plain between the bluffs at this point is approximately 10 miles. Studies were limited almost entirely to the river-facing bluffs.

The order of procedure was first to determine as nearly as possible the usual order of succession, or successions, on the river bluffs. With this as a background, study was then directed so as to determine the differences, if any, that existed between the successions on

bluffs on opposite sides of the river. Steps were then taken to find an explanation for the phenomena.
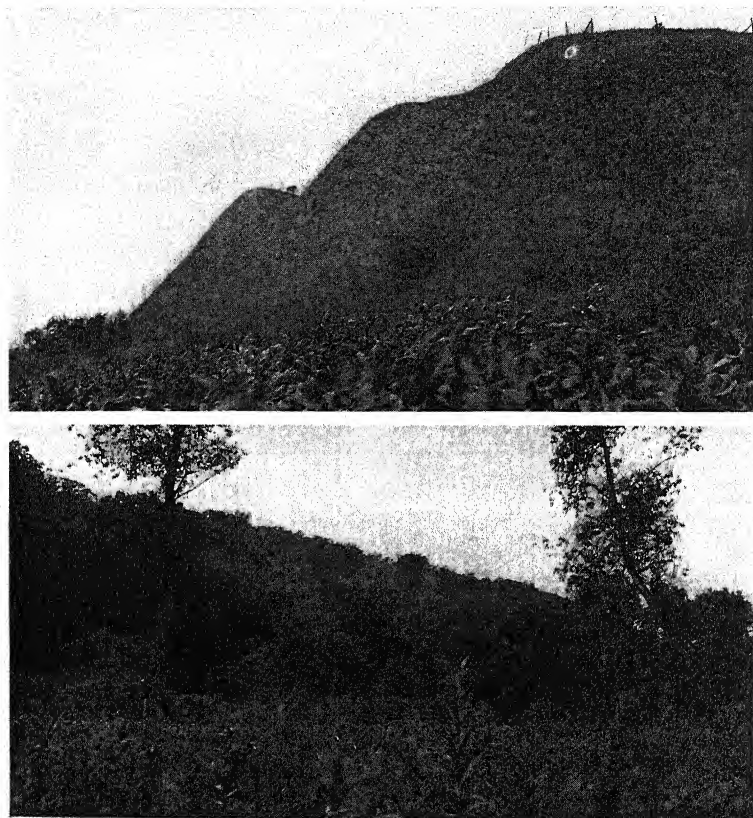
FIGS. 1, 2.—Fig. 1 (above), west-facing treeless bluffs on Iowa side of Missouri River; fig. 2 (below), east-facing forest-covered bluffs on Nebraska side.

Plant succession

The plant associations of the Missouri River bluffs have been variously described by different investigators. POUND and CLEMENTS (7) for Nebraska, in considering the river bluff formation, speak of the red oak-hickory association, the bur oak-elm-walnut association, and the linden-cedar-ironwood association. POOL, WEAVER, and JEAN (6) describe the "communities of the region about Peru in the

order of their place in succession as the bur oak-yellow oak type, the black oak-hickory type, the red oak type, and the linden-ironwood type." In actual practice it is impossible to describe the associations of any limited area of the bluffs by means of a general formula, because conditions of habitat permit the invasion of other species, or exclude certain of those which are characteristic of the associations, so as to make the type both variable and heterogeneous. For purposes of comparison, it has been found best to use a succession in which replacement takes place in the following order: bare soil, grassland, shrub association, bur oak-yellow oak association, red oak-black oak-hickory association, elm-ash-walnut association, linden-ironwood association. An elm-ash-ironwood association is prominent in some places and frequently takes the place of the elm-ash-walnut group. In this study, where observations were confined almost entirely to the river-facing bluffs, even these types failed in some instances.

Bare soil

In the Missouri River bluff region, the only natural agency that can produce a bare surface, devoid of vegetation and of disseminules for the propagation of plants, is erosion. Considerable erosion in these bluffs has come as the direct result of river action. In meandering over its floodplain, the Missouri River has cut hard on one bank and then on the other, producing high bluffs that were almost vertical at the time of their formation. At the present time, although the river is cutting hard on its right bank, little erosion is taking place at the base of the bluffs owing to its action. With the exception of a strip north of Nebraska City, the river is confined entirely to cutting its floodplain in the area studied.

Erosion, due to running water, has contributed greatly to the present topography of the bluffs. River action, however, is the only means that produces a surface where plants are entirely absent. Soil that is exposed by the river has been covered so long that any seed that could possibly still be present has long since lost its viability; hence the only means by which these areas are populated is through the agencies of plant distribution.

GRASSLAND ·ASSOCIATION

The possibility of fixing any plant as an absolute pioneer on these clay surfaces is remote. Conditions are not exacting enough to limit the number of plants that may serve as pioneers, while slump action
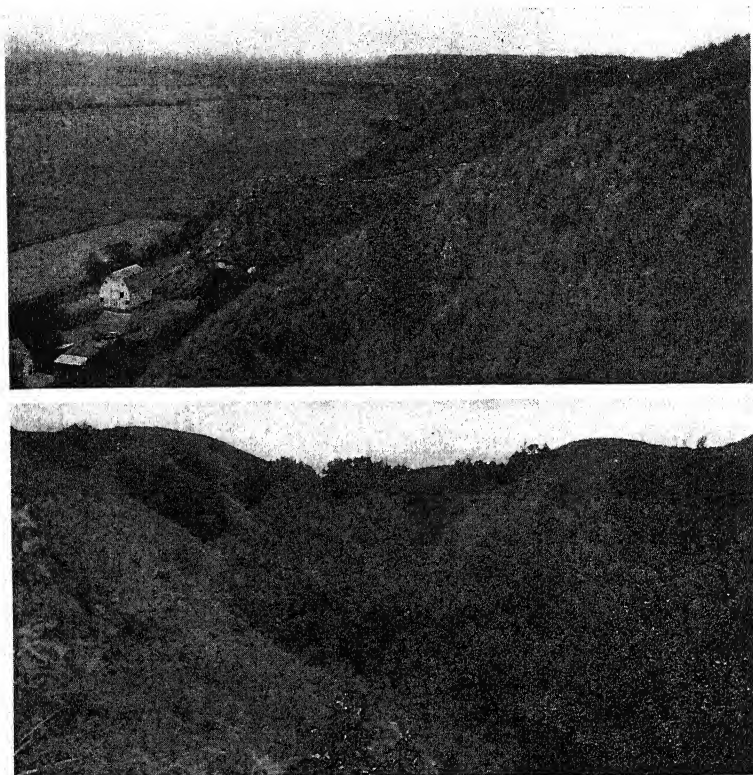
FIGS. 3, 4.—Fig. 3 (above), Iowa bluff showing typical grassland; floodplain of Missouri River in upper left; fig 4 (below), transition from grassland to shrub and timber associations on Iowa bluffs.

may introduce species that are entirely foreign to the situation. It is by this means that the great majority of eroding bluffs are finally "captured." The type that follows "capture" may well be classified as grassland, since grasses predominate in number of individuals so as to give character to the landscape (fig. 3). The usual pioneers that introduce this type are *Taraxacum officinale, Melilotus alba, M.*

officinalis, Aquilegia canadensis, Verbascum thapsus, and *Rumex crispa*. Later *Stipa comata, Glycyrrhiza lepidota, Petalostemum purpureum, P. multiflorum, Yucca glauca, Cirsium* sp., and *Liatris squarrosa* make their appearance, and a typical prairie flora results.

SHRUB ASSOCIATION

It is here that the transition from prairie grassland to timberland takes place. The association is usually found at the crest of the bluffs and bordering many of the patches of timber that are found on the more protected slopes (fig. 4). Many of the ravines in the prairie grassland association are given over to a shrub association. This is characterized by a low growth of woody forms, and may vary from an open stand of sumac on the prairie to a thick impenetrable tangle of woody forms in the gulches that have been formed by erosion. The more prominent species are *Rhus typhina, R. glabra, Cornus asperifolia, Prunus americana*, and *Crataegus* sp. Not all of these species are found in one place by any means; more frequently a single species is dominant within the limits of any particular area.

BUR OAK-YELLOW OAK ASSOCIATION

This is the association that usually encroaches upon the prairie, either with or without the aid of the shrubby forms. The growth is open and frequently the trees do not touch, so that the characteristic growth form is a tree with a comparatively low spreading and well rounded crown. *Quercus macrocarpa* and *Q. muhlenbergii* are the dominant species. The undergrowth normally consists of a layer of shrubby forms, such as *Corylus americana, Symphoriocarpos symphoriocarpos, Prunus americana, P. virginiana, Rhus glabra*, and *Cornus asperifolia*. When the canopy is less open the undergrowth may change to one in which *Ribes gracile, R. occidentalis, Psedera quinquefolia, Rhus toxicodendron, Vitis vulpina*, and *Smilax hispida* are dominant. The herbaceous layer is variable, and may consist of grasses such as *Andropogon scoparius* and *A. furcatus*, in combination with *Oenothera laciniata, Monarda fistulosa*, and various species of *Solidago;* or it may be practically devoid of herbaceous species if the shrub formation above is dense enough.

BUR OAK-BLACK OAK-HICKORY ASSOCIATION

This association is made variable by a considerable number of intruders that frequently appear. *Quercus macrocarpa*, *Q. velutina*, and *Carya ovata* give character to the association. It is not at all uncommon, however, to find specimens of *Q. rubra*, *Fraxinus americana*, *Juglans nigra*, *Carya porcina*, *Celtis occidentalis*, *Gymnocladus dioica*, *Ostrya virginiana*, *Cercis canadensis*, and *Juniperus virginiana*. The most important lianas are *Smilax hispida*, *Vitis vulpina*, and *Psedera quinquefolia*. Other climbers occur, principally in the secondary layer, such as *Clematis pitcheri* and *Echinocystis lobata*. The secondary layer nearly always consists of seedlings of the type that makes up the association. Shrubs are not numerous as a rule, and when they do occur it is usually as a result of some disturbance of the surrounding vegetation. The herbaceous layer is represented by such forms as *Viola* spp., *Aquilegia canadensis*, *Claytonia virginica*, *Phlox divaricata*, *Erythronium albidum*, *E. americanum*, and *Erigeron philadelphicus*. With the disappearance of these forms and the advent of drier conditions, the flora changes to one in which *Verbascum virgatum*, *Helianthus annuus*, *Abutilon theophrasti*, *Dentaria* sp., *Cryptotaenia canadensis*, *Osmorrhiza longistylis*, *Cassia chamaecrista*, *Sanicula marylandica*, *Mentha piperita*, and *Eupatorium maculatum* are prominent.

ELM-ASH-WALNUT ASSOCIATION

This association is even more variable than any that has yet been described. The dominant trees are *Ulmus fulva*, *Fraxinus americana*, and *Juglans nigra*. As in the preceding group, *Celtis occidentalis*, *Gymnocladus dioica*, *Ostrya virginiana*, and *Juniperus virginiana* may appear. Specimens of hickory are rarely found and oaks are also infrequent. *Staphylea trifolia*, *Amelanchier canadensis*, and *Tilia americana* make their appearance in the more mesophytic localities. *Prunus serotina* is frequently found in this admixture although it may occur in the preceding association.

LINDEN-IRONWOOD ASSOCIATION

This is the climax forest of the bluff region. The characteristic species are *Tilia americana*, *Ostrya virginiana*, *Fraxinus pennsyl-*

vanica, and *Morus rubra.* Frequently the last two species are entirely absent. The formation is characterized by tall straight trees with compact close fitting tops that provide a deep shade. A secondary layer of small trees and shrubs may or may not be preent. When it is preesent it usually consists of seedlings of *Tilia americana* and *Ostrya virginica* in combination with *Cornus stolonifera, C. asperifolia, Rhamnus lanceolata,* and *Symphoriocarpos symphoriocarpos. Rhus toxicodendron, Psedera quinquefolia, Smilax hispida,* and *Clematis virginiana* occur abundantly. The mesophytism of the situation is revealed by the presence of such forms as *Impatiens biflora, Claytonia virginica, Smilacina racemosa, Polygonatum biflorum, Galium aparine, Sanguinaria canadensis, Orchis spectabilis, Podophyllum peltatum,* and *Adiantum pedatum.* The linden-ironwood association is found at its highest development only in sheltered ravines at the base of the bluffs.

Bluff successions compared

The foregoing description has been given to provide a basis for the comparison of the bluff associations and their succession on opposite sides of the river. Points of difference and of similarity were brought out by observations, and measurements taken systematically and at random in the various associations. An exhaustive experimental study requiring considerable time will be necessary before the complete set of factors responsible for the differences can be determined.

Rate of succession

In considering data that have been brought out by a study of the comparative areas of grassland and of timberland on the two sides of the river, it is apparent that the rate of succession is much faster on the Nebraska side. Within equal periods of time a much greater amount of bluffland has become forested on the Nebraska side of the river than on the Iowa side. (This statement is made with respect to the river-facing bluffs only.) Protected bluffs and sheltered ravines behind the first range of bluffs bordering the floodplain show stages of vegetation as far advanced as those on the Nebraska side. Thus there is no doubt as to the possibility of reaching the climax of the area on the Iowa side.

An index of the rate at which succession takes place on opposite

sides of the river is provided by the secondary successions observable at various places. All of these seem to indicate that any disturbance of the natural vegetation remains visible longer on the west-facing than on the east-facing slopes.

NUMBER OF INDIVIDUALS AND VARIETY OF SPECIES

By means of belt transects 8 m. wide and quadrats 10 m. square it was found that the Nebraska bluffs excel both in number of species of trees and in number of individuals. The greater number of species is brought about by the appearance of intruders in the associations common to both sides of the river. Some of these invading species have already been mentioned in the description of the associations.

A comparison of undergrowth in the various woodlands would seem to indicate that there is a difference in density and number of species, but it is possible that grazing and biotic disturbances have rendered such data unreliable.

POSITION ON BLUFFS

It has been determined that corresponding associations are higher on the Nebraska bluffs than on the Iowa bluffs. In many cases the bur oak-yellow oak association, which has reached the crest of the east-facing bluffs, is to be found only half way up the slope on west-facing bluffs (fig. 5). In other situations it is confined entirely to the ravines or to the sheltered gulches on west-facing bluffs. In progressing down the bluff, the whole range of associations may be passed through until the linden-ironwood association is reached at the bottom. More frequently, however, where the bur oak formation is found only part way up the bluff, the base of the bluff is reached before the linden-ironwood association is encountered; conditions have not reached the mesophytism that will support such a forest.

A noteworthy feature of the Iowa bluffs is the telescoping of associations. Within a comparatively short distance all of the woodland associations may be encountered. The change from the bur oak-yellow oak association to the linden-ironwood climax has been observed within a space of 30 m., making a separation of associations

into distinct units an impossibility. This indicates a quick change from the mesophytic to the xerophytic, a phenomenon which is not nearly so evident on any of the Nebraska bluffs.
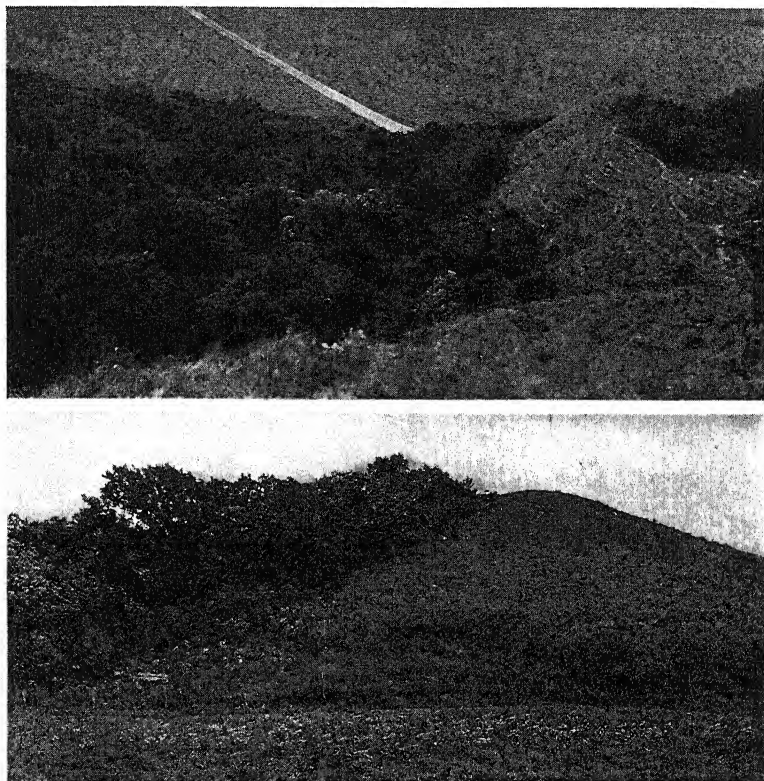
FIGS. 5, 7.—Fig. 5 (above), forest at crest of bluff on east-facing slope, half way up west-facing bluff (wind blows from left to right); fig 7 (below), Iowa bluff with forest growing in area protected from prevailing winds (wind blows from right to left).

COMPARISON OF LINDEN-IRONWOOD CLIMAX AREAS

As a result of measurements of area and a tabulation of species, taken systematically and at random, it has been found that a greater area has reached the linden-ironwood climax on the Nebraska side than on the Iowa side. Incidentally this is an indication that the rate of change is greater on east-facing than on west-facing slopes.

It is interesting to note that where the bluffs take an east and

west trend at one point on the Iowa side, thus providing a north-
facing slope next to the floodplain, development of vegetation is
much the same as that on the Nebraska side of the river. The sig-
nificance of this will be seen when the cause of the difference in rate
of succession on the two opposing sets of bluffs is taken into con-
sideration.

TREELESSNESS OF WEST-FACING BLUFFS

It is doubtful whether any one factor could be important enough
to cause such a striking difference in appearance in two series of
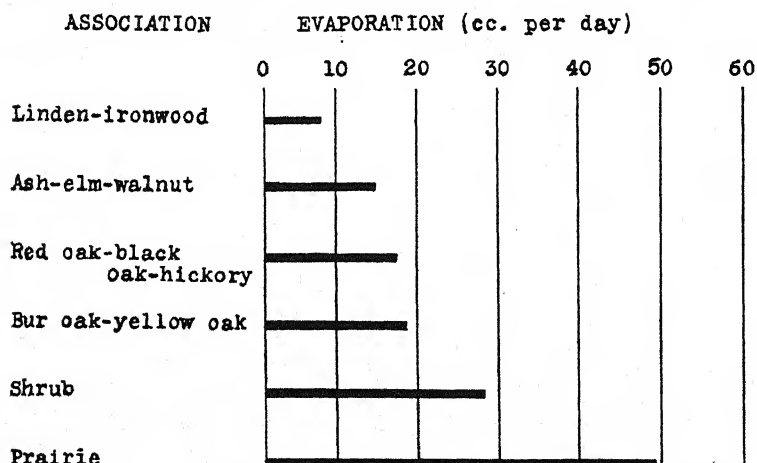
FIG. 6.—Average daily evaporation from atmometers in various associations on
Missouri River bluffs.

bluffs such as have just been described. On the other hand, it seems
probable that one factor has been responsible to an extent greater
than that of all other factors combined in bringing about the vegeta-
tional complex that now exists on the slopes of the Iowa and Nebras-
ka river bluffs. That factor is evaporation, caused by the prevailing
winds.

In the region studied, the prevailing winds from April 1 to October
1 are from the south and southwest, and from October 1 to April 1
from the northwest. East-facing slopes are protected from the dry-
ing influence of these winds, while west- and southwest-facing slopes
are windswept at all seasons of the year. Atmometer readings (fig.

6), taken from standardized spherical atmometer cups devised by LIVINGSTON (4), show that the rate of evaporation is almost twice as great on west-facing slopes where the wind strikes, as on east-facing slopes where protection is afforded. The amount of evaporation increases as one passes from the linden-ironwood association toward the bur oak-yellow oak association. When the shrub and grassland associations are reached the rate is almost twice that of the bur oak association, and nearly six times that of the linden-ironwood association. Rates of evaporation in similar associations on opposite sides of the river were nearly the same.

Further evidence in support of the evaporation theory is furnished by the observation that almost without exception, on both the Nebraska and the Iowa sides of the river, woodland vegetation ceases abruptly when the crest of the bluffs is reached. The change from forest to prairie or to shrub associations, when present, is so sharp that it is almost diagrammatic (fig. 7). In every case the east-facing slopes are covered with woodland while the west-facing slopes are bare of trees.

Still further evidence in support of this idea was brought out by the observation that apparent exceptions to the rule, west-facing bluffs covered with timberland, were protected by other bluffs to the windward. It would appear, therefore, that the explanation of the treelessness of west-facing bluffs in general is due, directly or indirectly, to the effects of a high rate of evaporation, caused by the prevailing winds during the growing season.

FUTURE STUDIES

The writer hopes to make a thorough and systematic study of all the factors at play in the bluff region described. The work of previous investigators has indicated the method of procedure. LIVINGSTON (5) has shown that there is a correlation between the evaporating power of the air and the growth of plants during the months in which frost is absent. WEAVER (9) believes that differing rates of evaporation in various plant formations are important factors in causing plant succession. FULLER (2), studying environmental factors on the sand dunes in northern Indiana, states that "differences in the rates of evaporation in the various plant associations studied

are sufficient to indicate that the atmospheric conditions are efficient factors in causing succession." It would seem worth while, therefore, to make careful measurements of atmospheric conditions over a wide area on the Iowa bluffs, in order to determine whether plant succession might be retarded or even made impossible in the presence of a high rate of evaporation.

Further studies will be necessary for a complete explanation of the treelessness of the Iowa bluffs. WARMING (8) states that "the absence of trees from many places on the earth is mainly due to wind," but does not go into detail. WEAVER and CLEMENTS (10) mention the retarding effects of increased transpiration on trees and further state that the drying effect of wind may result in the death of trees. BRYAN (1) suggests a point of attack through a study of the relation of the various associations to ground water level. It is possible that lowering of the water table, due to ravine cutting, has made it difficult for trees to grow on the west-facing slopes. This in turn calls attention to the great amount of erosion that has taken place on the west-facing slopes. GEIGER (3) has found that slopes facing the wind get more precipitation per unit of surface than slopes turned away from the wind. It is possible then that the prevailing winds have acted directly, through increasing the evaporation rate, and indirectly through influencing the topography over centuries, in producing treeless slopes on the west-facing bluffs of the Missouri River.

Summary

1. Study of the river-facing bluffs of the Missouri River was undertaken to gain a more accurate concept of the differences that appear to exist between the opposing bluffs.

2. In order to provide a basis for discussion, a brief description of the order of succession on these river bluffs and their plant associations is given.

3. The order of succession was found to be the same on opposite sides of the river.

4. The rate of succession is faster on east-facing bluffs.

5. East-facing bluffs excel both in number of individuals and in variety of species.

6. Corresponding associations are found higher on east-facing than on west-facing bluffs.

7. A greater area is occupied by the linden-ironwood climax on east-facing bluffs than on west-facing bluffs.

8. These differences, as well as the treelessness of west-facing bluffs, appear to result from the high rate of evaporation caused by prevailing winds during the growing season.

9. It is hoped to study in detail the environmental factors in the various plant formations and associations on the Missouri River bluffs.

The writer is grateful to Professors H. C. COWLES and G. D. FULLER of the University of Chicago for encouragement and criticism during the progress of this investigation.

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NEW OR OTHERWISE NOTEWORTHY COMPOSITAE. VI

EARL EDWARD SHERFF

Bidens mildbraedii sp. nov.—Herba perennis e radice lignescenti, erecta, probabiliter 7–12 dm. alta, ramis fastigiato-erectis, caule ramisque atropurpureis, glabris, angulato-teretibus vel subtetragonis. Folia opposita, petiolo adjecto 0.7–1.7 dm. longa, nunc indivisa, linearia, 2–7 mm. lata, superne ad apicem acerrimum sensim elongato-attenuata inferne in petiolum marginatum 2–3.5 cm. longum angustata; nunc supra petiolum utrinque anguste 1-lobata lobo tenuiter lineari 1–3 cm. longo et 0.5–2 mm. lato; nunc pinnatim 3- vel 5-partita, foliolis linearibus 1–4 mm. latis, inferioribus interne saepe rursus 1-lobatis, petiolo tenui 2–4 cm. longo; laminis segmentisve membranaceis, supra glabris infra glabris vel aegre adpresso-hispidis, marginaliter parce subrevolutis sed hispido-ciliatis. Capitula corymbosa, tenuiter pedunculata pedunculis superne dense pilosis pilis patentibus, radiata, pansa ad anthesin circ. 2.8–3.4 cm. lata et 8–12 mm. alta. Involucri bracteae inferne valde superne aegre hispidae, exteriores 8–10, lineari-oblongae, apice indurato-nitidae et saepius obtusae, 6–14 mm. longae; interiores ovatae, 6–9 mm. longae. Flores ligulati circ. 8, sicci atroflavi, lineari-oblongi, circ. 1–1.3 cm. longi et circ. 2–3.5 mm. lati, apice profunde acriterque lobulati lobulis 1–3 mm. longis. Paleae lineari-oblongae, subtiles, apice obtusae, demum 7–9 mm. longae et circ. 1 mm. latae. Achaenia lineari-oblonga, plana, brunneo-atra, unaquaque facierum circ. 8-sulcata, faciebus marginibusque erecto-hispida, nunc parva, corpore 4.5–6 mm. longa et circ. 0.6–0.8 mm. lata, apice valde erecto-setosa et biaristata aristis erectis vel suberectis basaliter sursum hispidis aliter nudis 0.8–1.3 mm. longis; nunc majora, corpore 7–9 mm. longa et 1–1.6 mm. lata, apice subsetosa et biaristata aristis patentibus inaequaliter 0.5–1 mm. longis, nudis vel basaliter rarius erecto-setosis.

Specimens examined: *Mildbraed* 9777, alt. about 1000 m., Buar, Kamerun, July, 1914 (type in Herb. Kew); *idem* 9386, *eodem loco*, May, 1914 (Herb. Kew).

The two sheets examined are of material collected two months apart. The first sheet has two plants. These were collected in May. One has a sterile, leafy stem about 2.7 dm. tall; the other has a sterile stem about 2.4 dm. tall and a fertile stem about 6.2 dm. tall, bearing three fruiting heads. The leaves are of the more compound type and offer a strong superficial resemblance to those of the North American *Coreopsis delphinifolia* Lam. The exterior involucral bracts are equal to or a fourth or third shorter than the interior ones. The achenes are the larger ones of the Latin description.

The second or type sheet bears material of a much better developed plant (or plants). This was collected in July. About two dozen heads are present. The leaves are simple or laterally 2-lobed. The exterior involucral bracts tend greatly to exceed the interior ones. The achenes are the smaller ones of the Latin description.

As these differences appear to be of a seasonal nature, it seems inadvisable to attempt any varietal distinction of one form from the other.[1]

Bidens rhodesiana sp. nov.—Herba erecta, perennis, ramosa, verisimiliter circ. 8–10 dm. alta, breviter denseque hispida, caule ramisque tetragonis, internodiis elongatis. Folia opposita subsessilia vel petiolata petiolis alatis usque ad circ. 1.3 cm. longis, petiolo adjecto 3–7 cm. longa (inferiora non visa), membranacea sed rigidula, circumambitu nunc late nunc anguste deltoideo-ovata, pinnata, foliolis lateralibus 1–2 jugis ovatis terminali ovato lanceolatove, omnibus dentatis dentibus obtusis vel etiam orbiculatis sed acriter mucronulatis. Capitula pauca, longe tenuiterque pedunculata, radiata, pansa ad anthesin 5–6 cm. lata et circ. 1–1.2 cm. alta. Involucri bracteae dorsaliter hispidae exteriores circ. 8, nunc lineari-oblongae nunc etiam oblongo-ovatae, apicaliter obtusae, circ. 4–8 mm. longae; interiores ovato-lanceolatae 9–12 mm. longae. Flores ligulati circ. 9, flavi, ligula oblongo-oblanceolati, apice obscure 3-denticulati, 2–2.5 cm. longi. Paleae lineari-oblongae, circ. 7–8 mm. longae. Achaenia plana, atro-brunnea, lineari-oblonga, basim ver-

[1] I am greatly indebted to Dr. Arthur W. Hill, Director of the Royal Botanical Garden of Kew, for sending me the *Mildbraed* specimens for study.

sus paulo angustata, faciebus glabra, unaquaque facierum circ. 8-sulcata, marginibus sursum setosa, apice sursum setosa ac biaristata aristis tenuibus glabris plerumque 1–1.5 mm. longis.

Specimens examined: *A. J. Teague* 226, in the Odzani River Valley, District of Manica, Division of Umtali, Southern Rhodesia, 1914 (type in Herb. Kew).

Through some of its leaves this species approaches closely *B. ukambensis* S. L. Moore. From that species it differs easily, however, in its much broader flowering heads, smooth-faced biaristate achenes, etc.

Bidens setigeroides sp. nov.—Herba annua, erecta, laxe hispida, caule subtetragona, ramosa ramis suberectis, 3–9 dm. alta. Folia 2–3-pinnatisecta, breviter petiolata petiolis latis usque ad 1 cm. longis, petiolo adjecto usque ad circ. 7 cm. longa, segmentis membranaceis, acriter apiculatis et interdum in setas breves desinentibus, plerumque 1–2 mm. latis. Capitula subcorymboidea, tenuissime pedicellata pedicellis pubescentibus 2–6 cm. longis, numerosa, radiata, pansa ad anthesin ± 1.2 cm. lata et circ. 6 mm. alta, demum circ. 8–9 mm. diametro. Involucri bracteae plus minusve hispidae, exteriores plerumque 6, anguste lineares vel lineari-spathulatae, apicaliter obtusae, 2–3 mm. longae; interiores oblongo-ovatae, ± 3.5 mm. longae. Flores ligulati circ. 6, flavi, ligula oblongo-ovati, apice circ. 3-dentati, circ. 5–6 mm. longi. Paleae nitidae, glabrae, oblongae, subobtusae, apicem versus aurantiacae, 3–6 mm. longae. Achaenia oblanceolato-linearia vel late oblanceolata, nigra, exalata, non manifeste sulcata, dorso convexa ac glabrata, ventre plana vel concava ac costa mediana erecte setosa, marginibus crassis erecte ciliata, apice truncato exaristata sed erecte setosa, 2.2–3.5 mm. longa et 0.5–1.2 mm. lata.

Specimens examined: *Mr. J. D. Snowden* 411, growing 1–3 feet high in short-grassland at altitude of 5000 feet, Wallasi, Bukedi, Uganda Protectorate, British East Africa, October 17, 1916 (Kew).

Differs from *Bidens setigera* of Abyssinia and Eritrea in the less setigerous foliar teeth, the fewer exterior bracts of the involucres, the exaristate, smoother achenes, etc. It approaches in general appearance the habitally similar *Coreopsis negriana* Sherff of Galla-

land, but differs from that species in the smaller flowering heads, the setose, exalate achenes, etc.

BIDENS DIELSII **medusoides** var. nov.—A specie involucri bracteis exterioribus 14–20 (plerumque circ. 16–18), lineari-subulatis, 0.8–1 (rarius usque ad 2) cm. longis, inferne 0.6–1 (raro usque ad 1.5) mm. latis differt.

Specimens examined: *T. D. Maitland* 1004, frequent in grassland at altitude of 5000 feet, Wasa, region of Mt. Ruwenzori, Tropical East Africa, Dec. 15, 1925 (Kew).

In the young heads (much before anthesis) the exterior bracts are twice as long as the disk, with a resulting fanciful resemblance of these heads to the *medusa* stage of many of the Coelentera in the animal kingdom.

BIDENS SETIGERA **lobata** var. nov.—Folia circumambitu oblongo-lanceolata vel anguste deltoideo-lanceolata, pinnatim plus minusve incisa vel etiam subtripartita, inferiora non visa alia 2–5 cm. longa, dentibus perspicue setigeris.

Specimens examined: Cultivated in garden of *J. Veitch and Sons*, 1908, from material collected by *Captain Diespecker* in British East Africa (Kew, 2 sheets).

The first type sheet has the six flowering heads measuring about 2.2–2.7 cm. across, with about 12–16 exterior bracts. The second type sheet has likewise six flowering heads, but these are mostly 4–4.5 cm. across and the exterior bracts number 16–22. These differences are construed as representing different states of luxuriance due to conditions of cultivation. The difference in general aspect due to the different amounts of foliar dissection is great, but such a difference is known in many other species of *Bidens* and does not appear to justify specific segregation.

Bidens skottsbergii sp. nov.—Frutex glaberrimus, verisimiliter 1 m. altus, ramosus. Folia opposita, tenuiter petiolata petiolis circ. 1–3 cm. longis, petiolo adjecto 8–13 cm. longa, pallidiora, membranacea, indivisa, oblongo-ovata, subgrosseserrata (unico latere saepius 12–18-serrata), apice subobtusa, basi late cuneata, principalia 3.5–5 cm. lata. Capitula pauca, laxe paniculata, pedunculata pedunculo subtenui usque ad 9 cm. longo, radiata, pansa ad anthesin 3–4 cm. lata et 9–12 mm. alta. Involucri bracteae exteriores circ. 8–10, glaberri-

mae, nunc late lineari-oblongae nunc oblongo-spathulatae, apicaliter obtusae, demum saepe patentes, 4–6 mm. longae; interiores oblongo-lanceolatae paulo longiores, apicaliter pubescentes. Flores ligulati circ. 8, flavi, ligula nunc oblongi nunc obovati, apice truncato 2–5-dentati et saepe subprofunde scissi, 1.7–2 cm. longi et 6–8 mm. lati. Achaenia nigra, plana, lineari-oblonga, unaquaque facierum plus minusve 8-striata, corpore glabro 8–10.5 mm. longa et circ. 1.35 mm. lata, lateribus marginata sed non vere alata, apice biaristata (aliter glabra) aristis subvalidis, stramineis brunneisve, retrorsum multi-hamosis, 1.5–2 mm. longis.

Specimens examined: *Carl Skottsberg* 2092, near Puna Road, south of Hilo, Isl. Hawaii, Sept. 30, 1926 (type in Herb. Bot. Gard. Göteborg).

Most closely related to *Bidens populifolia* Sherff (of the Island of Oahu), from which it differs in its oblong-ovate, basally wide-cuneate leaf blades, its broader, shorter, and more oblong achenes, these lacking erect apical setae between the aristae and having the aristae more densely and regularly retrorse-hamose.

Heterosperma nanum (Nutt.) comb. nov.; *Microdonta nana* Nutt., Trans. Amer. Phil. Soc. ser. II, **7**:370. 1841.—BENTHAM and HOOKER (Gen. Pl. **2**:384. 1876) noted this species ("ex specimine parvo et auctoris descriptione videtur *Heterospermi* species") but did not make a definite new combination.

Isostigma molfinianum sp. nov.—Herba perennis, basi fruticosa, erecta suberectave, glaberrima, circ. 1.5 dm. alta, inferne simplex ac valde foliosa internodiis plurime circ. 2–4 mm. longis, superne corymboideo-ramosa ramis erectis gracilibus angulatis. Folia sub-coriacea, inferiora lineari-oblanceolata, apicaliter plerumque 2–3-furcata dentibus apicaliter mucronatis usque ad circ. 8 mm. longis, inferne sensim in petiolum saltem 1–2 cm. longum angustata, petiolo adjecto 2.5–4 cm. longa et (sub dentibus) circ. 2.5–3.5 mm. lata; superiora anguste oblongo-linearia, apicaliter plerumque 2–3-furcata dentibus parvis rarissime integra acutaque, basaliter aegre angustata. Capitula (±10 in unico specimine) corymboideo-paniculatim dis-posita, aegre radiata, pansa ad anthesin circ. 8–11 mm. alta et circ. 1.2–1.6 cm. lata. Involucri bracteae exteriores circ. 9 vel 10, lanceo-latae, tergo hispidae, marginaliter albido-hyalinae, circ. 3–4 mm. longae et circ. 0.6–0.75 mm. latae, adpressae vel superne reflexae;

interiores late oblongo-lanceolatae, tergo glabratae, marginaliter brunneo-hylinàe, paulo longiores. Flores ligulati forsitan 8–10, ligula (4–6 mm. longa) late lineari-oblongi, circ. 5–6-striati, apice saepe dentati, tubo circ. 2 mm. longo; stigmatibus capilliformibus ligulam manifeste superantibus; ovario plano obovato, faciebus brunneis glabrato, apice biaristata aristis tenuibus patentibus circ. 5 mm. longis basaliter subsursum hispidis aliter calvis. Paleae lineares, superne coloratae et saepe tortae, 5–7 mm. longae. Flores tubulosi cylindrico-urceolati, corolla (circ. 6 mm. longa) profunde (fere usque ad medium) 4-fidi, lobis elongate lineari-lanceolatis; antheris stig-matibusque valde exsertis. Achaenia matura deficientia; ovariis planis, anguste obovatis, brunneis, faciebus pilis adpressis suberectis-que plus minusve hispidis, apice perspicue biaristatis; aristis tenui-bus, patentibus, brunneis, 4–5.5 mm. longis, corollae tubum gracilem saltem duplo superantibus, basaliter aegre sursum hispidis aliter levibus vel apicem versus raro 1 vel 2 hamis retrorsum hamosis.

Specimens examined: *G. Bodenbender*, marshy place ("totoral") at Quebrada, Province of La Rioja, Argentina, February, 1896 (type in Herb. Gray).

This species was unknown to me at the time my revision of the genus *Isostigma* was published (Bot. Gaz. 81:241–257. 1926). The type sheet bears two small specimens, each about 1.5 dm. tall. They are nearest to *Isostigma herzogii* Hassl., of which only the type, a fragment likewise about 1.5 dm. tall, is known to me. *I. molfi-nianum* differs in having the stems thicker below, much shorter internodes and consequently many more leaves, practically all the leaves (excepting only the bracts of the inflorescence) apically 2–3-furcate instead of entire, the ligules 4–6, not 9–11, mm. long, the anthers more conspicuously exserted (often suggesting an extra corolla), the disk corollas more deeply lobed (the narrow sinuses often reaching almost half-way down to the slender tube), the aris-tae of all the ovaries much longer (4–5.5 not 1.5–2 mm. long) and those of the disk corollas more than twice as long as the corolla tube (instead of barely equaling it), etc. The florets all are somewhat discolored with age, mostly appearing at present somewhat purplish-brown. One ligule, however, has a more yellow-brown color.

For the privilege of examining the type I am indebted to Dr. Ivan M. Johnston of Gray Herbarium. He had received it from

Sr. José Molfino, "one of the foremost botanists of Argentina." It is a pleasure to commemorate Sr. Molfino in the choice of a trivial name.

In a recent article (BOT. GAZ. 89:371–373. 1930) I presented a key to and conspectus of all known South American species of *Coreopsis*. It has seemed desirable to publish at this time corresponding treatments for the known African species of that genus. Accordingly they are presented herewith. Authentic material of *Coreopsis aspilioides* Baker (Kew Bull. **1898**:153. 1898) and *C. chevalieri* O. Hoffm. & Muschl. (Bull. Soc. Bot. France 57, Mem. VIII. 118. 1910) has not been studied and hence those two species are omitted. All other published species excluded here are those which have been found properly referable elsewhere, particularly to *Bidens*.

Clavis plantarum africanarum Coreopsidis

a. Achaenia valde obcompressa, marginibus saepissime tenuia alatave

  b. Aristae achaeniorum filiformia ac quam corporibus breviora, vel deficientes

    c. Plantae annuae (vel verisimiliter annuae)

      d. Folia superne hispida

        e. Achaenia (alis inclusis) circ. 3 mm. lata..30. *C. oligoflora*

        e. Achaenia (alis inclusis) 4–5 mm. lata...31. *C. mattfeldii*

      d. Folia superne glabra vel glabrata

        e. Achaenia exaristata.................24. *C. negriana*

        e. Achaenia biaristata

          f. Foliorum caulinorum saepius 2–3-pinnatisectorum segmenta anguste linearia; capitulis pansa ad anthesin 2.5–5 cm. latis

            g. Foliorum segmenta plerumque circ. 1 mm. lata; involucro hispido..............29. *C. leptoglossa*

            g. Foliorum segmenta plerumque 2–4 mm. lata; involucro glabrato vel subglabrato...28. *C. feruloides*

          f. Foliorum 2-pinnatisectorum segmenta principalia oblongo-lanceolata; capitulis pansis ad anthesin 2.5–4 cm. latis....27. *C. prestinariaeformis* var. *incisa*

    f. Foliorum caulinorum integrorum laminae vel 3–5-partitorum segmenta late linearia; capitulis pansa ad anthesin 5–7 cm. latis.............39. *C. borianiana*

    f. Foliorum 3–5-lobatorum foliola ovata vel ovato-sublanceolata..............27. *C. prestinariaeformis*

c. Plantae perennes (vel verisimiliter perennes)

  d. Foliorum principalium laminae vel segmenta lanceolata vel latiora

    e. Foliorum segmenta dentata dentibus perspicue elongatis (saepe 1–1.5 cm. longis)............21. *C. mildbraedii*

    e. Foliorum segmenta diversa

      f. Foliorum dentes in setas elongatas desinentes.......
..............................11. *C. glaucescens*

      f. Foliorum dentes non setigerae

        g. Folia indivisa

          h. Folia scabra, saepius 10–16 cm. longa; involucro hispido.....................19. *C. scabrifolia*

          h. Folia glabra, 1.5–5 cm. longa; involucro glabro..
..............................9. *C. barteri*

        g. Folia divisa

          h. Achaenia exaristata

            i. Folia plerumque pinnata vel pinnatifida segmentis principalibus saepius late oblongo-lanceolatis vel ovatis, inter se saepe propinquis, supra scaberulis subtus tomentellis

            j. Caulis primo tomentellus mox glabratus...
..........................15. *C. whytei*

            j. Caulis perpetuo tomentellus.............
....................13. *C. pinnatipartita*

            i. Folia 2–3-pinnatisecta, foliolis vel segmentis principalibus saepius anguste oblongo-lanceolatis, glabris.................14. *C. lupulina*

          h. Achaenia biaristata

            i. Capitula pansa ad anthesin 5.5–9 cm. lata

              j. Folia pubescentia.............29. *C. bella*

              j. Folia faciebus glabra........18. *C. togensis*

      i. Capitula pansa ad anthesin usque ad 4 cm. lata

      j. Folia molliter denseque pubescentia; achae-
niis plumbeo-nigris minutis corpore tan-
tum 3–4 mm. longis.......16. *C. neumannii*

      j. Folia glabra vel subglabra

        k. Pauca folia divisa..........9. *C. barteri*

        k. Folia plerumque divisa

          l. Involucrum hispidum....12. *C. oblonga*

          l. Involucrum glabrum...10. *C. monticola*

d. Foliorum principalium laminae vel segmenta late vel
anguste linearia

  e. Foliorum dentes saepe in setas elongatas desinentes;
lamina indivisa, usque ad 2 dm. longa et 4–10 mm. lata
..............................20. *C. bracteosa*

  e. Foliorum dentes non setigerae

    f. Folia inferiora 1–2 dm. longa; involucri bracteae
exteriores circ. 12, lineari-subulatae, 1–1.5 cm. longae
.....................·...............4. *C. camporum*

    f. Folia inferiora raro 1 dm. longa

      g. Frutices

        h. Achaenia exaristata

          i. Segmenta ultima 2–3 mm. lata. 1. *C. elgonensis*

          i. Segmenta ultima multo angustiora..........
...........................2. *C. chippii*

        h. Achaenia biaristata..........3. *C. scopulorum*

      g. Herbae

        h. Capitula pansa ad anthesin 4–8.5 cm. lata

          i. Involucri bracteae exteriores elongatae, late-
raliter lobatae; ligulis sulphureis...........
..........................5. *C. ellenbeckii*

          i. Involucri bracteae exteriores normales; ligulis
aurantiacis..................17. *C. ochracea*

        h. Capitula pansa ad anthesin circ. 2–2.5 cm. lata

          i. Folia atro-viridia, segmentis plerumque 1.2–
2.5 mm. latis...............6. *C. lineariloba*

          i. Folia pallida, segmentis plerumque circ. 1
(raro 2) cm. latis...........7. *C. schimperi*

   b. Aristae achaeniorum subulata
      c. Herbae annuae
         d. Involucri bracteae interiores glabrae....26. *C. prestinaria*
         d. Involucri bracteae interiores pilis subplanis obtectae....
         ....27. *C. prestinariaeformis et* var. *incisa*
      c. Herbae perennes......................8. *C. macrantha*
a. Achaenia tantum moderate vel aegre obcompressa
   b. Achaenia exaristata; foliis indivisis spathulato-oblanceolatis
   ....23. *C. jacksonii*
   b. Achaenia biaristata aristis lanceolato-subulatis; foliis bipin-
   natipartitis................25. *C. pachyloma et* var. *inanis*

Conspectus plantarum africanarum Coreopsidis

   1. C. ELGONENSIS Sherff, Bot. Gaz. 80:374. 1925.—Uganda.
   2. C. CHIPPII M.B.Moss, Kew Bull. **1929**:196. 1929.—Southern
Sudan.
   3. C. SCOPULORUM Sherff, *loc. cit.* 88:302. 1929.—British East
Africa.
   4. C. CAMPORUM Hutch., Kew Bull. **1921**:381. 1921.—Northern
Nigeria.
   5. C. ELLENBECKII O. Hoffm., Engler Bot. Jahrb. 38:205. 1906.
—Eastern Abyssinia.
   6. C. LINEARILOBA O. Hoffm., *loc. cit.* 30:430. 1901.—South-
westernmost German East Africa.
   7. C. SCHIMPERI O. Hoffm., *loc. cit.* 38:205. 1906; *Microlecane
abyssinica* f. *elongata* Vatke, Linnaea 39:497. 1875.—Abyssinia.
   8. C. MACRANTHA Schz. Bip. *in* Walpers Repert. 6:163. 1846;
Verbesina macrantha (Schz. Bip.) A. Rich. Tent. Fl. Abyssin. 1:408.
1847; *Coreopsis macroptera* Schz. Bip. *in* Schweinf. Beitr. Fl.
Aethiop. 284. 1867; *C. prestinaria* f. *elatior* Vatke, Linnaea **39**:498.
1875; *Prestinaria macrantha* Schz. Bip. *ex* O. & H. *in* Oliver Fl.
Trop. Afr. 3:391. 1877.—Abyssinia.
   9. C. BARTERI O. & H. *in* Oliver Fl. Trop. Afr. 3:390. 1877; *C.
badia* Sherff, Bot. Gaz. **76**:90. 1923.—Northwestern Africa, from
Kamerun west to Togo and northward to Borgu.
   10. C. MONTICOLA (Hook. f.) O. & H. *loc. cit.; Verbesina* (*Presti-
naria*) *monticola* Hook. *f.*, Jour. Linn. Soc. **7**:200. 1864.—Kamerun.

A pilose variety of this species exists and has been recognized in herbaria by OTTO HOFFMANN, HUTCHINSON and DALZIEL, and others.

11. C. GLAUCESCENS O. & H. *loc. cit.* 389; *C. abyssinica* f. *latisecta* Vatke, Linnaea **39**:499. 1875 (*nomen subnudum*).—Abyssinia.

12. C. OBLONGA Sherff, BOT. GAZ. **76**:80. 1923.—Lake Tanganyika region, eastern equatorial Africa.

13. C. PINNATIPARTITA O. Hoffm., Engler Bot. Jahrb. **30**:432. 1901.—Nyassaland (British Central African Protectorate) northward into German East Africa.

14. C. LUPULINA O. Hoffm., *loc. cit.*—Southwesternmost German East Africa.

15. C. WHYTEI S. L. Moore, Jour. Linn Soc. **35**:348. 1902.—Nyassaland. Too close to *C. pinnatipartita.*

16. C. NEUMANNII Sherff, BOT. GAZ. **76**:85. 1923.—Southern Abyssinia.

17. C. OCHRACEA O. Hoffm., Engler Bot. Jahrb. **30**:431. 1901; Sherff, *loc. cit. et* pl. XIX; *C. cosmophylla* Sherff, *loc. cit.* **76**:90 *et* pl. IX, figs. *h-n.* 1923; *Bidens ochracea* (O. Hoffm.) Sherff, *loc. cit.* 158.—Northern Nyassaland northward through German East Africa into British East Africa.

18. C. TOGENSIS Sherff, Bot. Gaz. **76**:87. 1923.—Togo

19. C. SCABRIFOLIA Sherff, *loc. cit.* 86.—Belgian Congo.

20. C. BRACTEOSA Sherff, *loc. cit.* 88.—German East Africa.

21. C. MILDBRAEDII Muschler, Wiss. Ergebn. Deutsch. Zentr.-Afr.-Exped. 1907–1908, **2**:381. 1911.—Belgian Congo.

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C. JACKSONII var. ARTHROCHAETA Sherff, *loc. cit.* **88**:302. 1929.—British East Africa.

24. C. NEGRIANA Sherff, *loc. cit.* **90**:397. 1930.—Gallaland.

25. C. PACHYLOMA O. & H. *in* Oliver Fl. Trop. Afr. **3**:391. 1877; *C. involucrata* Schz. Bip. *in* Walpers Repert. **6**:163. 1846 (*non* Nutt., Jour. Acad. Phila. **7**:74. 1834); *Verbesina involucrata* (Schz. Bip.)

A. Rich. Tent. Fl. Abyssin. **1**:409. 1847; *Coreopsis callosa* Schz. Bip. *in* Schweinf. *et* Aschers. Enum. 284. Abyssinia.

C. PACHYLOMA var. INANIS Sherff, BOT. GAZ. **90**:386. 1930.— Abyssinia.

26. C. PRESTINARIA Schz. Bip. *in* Walpers *loc. cit.; Prestinaria bidentoides* Schz. Bip., *loc. cit.; Verbesina veris* A. Rich. Tent. Fl. Abyssin. **1**:407. 1847; *Coreopsis prestinaria* f. *typica et* f. *latisecta* Vatke, Linnaea **39**:498. 1875.—Abyssinia.

27. C. PRESTINARIAEFORMIS Vatke, *loc. cit.* 499; *C. pristinariaeformis* Vatke *ex* O. & H. *in* Oliver Fl. Trop. Afr. **3**:387. 1877 (sphalm); *C. heterocarpa* Chiov., Ann. Bot. Roma **9**:75. 1911 *(excl. Chiovendae plantam n. 1090 qua var. mea incisa est)*.—Abyssinia.

C. PRESTINARIAEFORMIS var. INCISA Sherff, BOT. GAZ. **90**:387. 1930.—Abyssinia.

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C. OLIGOFLORA var. ROBUSTA Sherff, BOT. GAZ. **90**:386. 1930. —Belgian Congo.

31. C. MATTFELDII Sherff, BOT. GAZ. **76**:83. 1923.—German East Africa.

32. C. BORIANIANA Schz. Bip. (*includ.* var. *cannabina* Schz. Bip.) *ex* Schweinf., Verhandl. Zool.-Bot. Ges. Wien **18**:684. 1868; *C. guineensis* O. & H. *in* Oliver Fl. Trop. Afr. **3**:390. 1877; *C. chrysopterocarpa* Chiov., Ann. Bot. Roma **9**:75. 1911.—Across northern Africa from Eritrea to Senegal (Sénégal) and reaching southward to Kamerun and Togo.

C. BORIANIANA var. MULTIPLEX Sherff, BOT. GAZ. **90**:385. 1930.—Togo.

CHICAGO NORMAL COLLEGE
CHICAGO, ILL.

DISTRIBUTION OF TOTAL NITROGEN IN REGENERATION OF THE WILLOW

P. A. DAVIES

(WITH FIVE FIGURES)

Introduction

The correlation between growth and available supply of carbohydrates and nitrogen has been studied by various workers. BUTLER, SMITH, and CURRY (1), with data secured from experiments on apple trees, came to the conclusion that vegetative awakening (bud development) is correlated with translocation of reserve nitrogen to the growing point. KRAUS and KRAYBILL (8) found that vegetative growth and reproduction in the tomato depend to a large extent upon an available carbohydrate-nitrogen relationship. They also found a descending gradient of total nitrogen from the apex to the base. HARVEY (5) reported that, in shoots of the apple, relatively great chemical differences exist between tip and basal areas. Nitrogen is more abundant at the apex; carbohydrates at the base. The results of MURNEEK (9) point to nitrogen as an immediate limiting factor affecting the influence of correlation in the tomato plant. The fruits monopolize almost all the available nitrogen; and in plants which are low in nitrogen, a single fruit will inhibit vegetative development. When the fruit is removed, vegetative development continues. Recently Miss REID (10, 11, 12) has produced valuable results on the carbohydrate-nitrogen relationship with seedlings of tomato, wheat, and squash. Her results indicate that increased nitrogen increases the growth of stems and leaves, and in the low-protein seedlings more than in the high-protein seedlings; and that an increased supply of carbohydrates increases root response, especially in the high-protein seedlings.

Regeneration is but a growth process, so the carbohydrate-nitrogen relationship should hold equally as well for regeneration as for growth. Miss HICKS (6) has applied the results obtained by KRAUS and KRAYBILL, HARVEY, MURNEEK, REID, and others to regenera-

tion in *Salix viminalis*. She states that active respiration seems to supply energy to expedite the translocation of nitrogen upward and the flow of carbon toward the base. Prior to growth, a lower C/N ratio is established at the apex and a higher C/N ratio at the base. Shoots develop in the area of lowest C/N ratio (apex) and roots in the area of highest C/N ratio (base). Her observations do not always agree with her conclusions. The nitrate-infused cuttings failed to develop, while in the carbohydrate-infused cuttings shoot development was greater than in the control. The writer's work shows the distribution of total nitrogen in the early stages of regeneration and development in *Salix nigra*. Some of the conclusions agree with those of Miss Hicks, while others do not.

Materials and methods

In studying regeneration, it is desirable to obtain material in which regeneration and development take place rapidly without the use of nutrient solutions. Under such conditions, the power of regeneration and development is invested solely in the cutting, and is in no way influenced by outside nutritive materials. *Salix nigra* was found to be favorable for this study, because shoot development and root regeneration take place rapidly, and polarity under normal conditions is very distinct.

For each experiment an entire willow shoot was used. It is necessary to know the total nitrogen gradient of the entire shoot, so that when cuttings are made, the gradient of each cutting is known. The gradient was obtained by making total nitrogen analyses at intervals along the entire shoot. The remainder of the shoot was then cut into 6-inch lengths. The cuttings were suspended, either in a normal or an inverted position, so that the lower half of each cutting was in well aerated distilled water, which was changed daily. The cuttings were kept in a dark chamber at 25° C.

The amount of material used for each analysis varied with the size of the cutting. In large cuttings, 1.5-inch pieces were used; in small cuttings, 2–3-inch pieces. The pieces were divided lengthwise into quarters. Opposite quarters were used for dry weight determination and the remaining quarters for total nitrogen analysis. By using the opposite quarters, the possible error was reduced. For

dry weight determinations, the material was placed in an oven at 102° C. and allowed to remain until a constant weight was obtained. Because there was only a small amount of root and shoot material available, more constant results were obtained by using the entire amount for total nitrogen on a dry weight basis, rather than dividing it as was done with the cuttings. The results given for roots and shoots are from dry weight materials. The total nitrogen analyses were made by the Kjeldahl-Gunning method. The results are shown in milligrams of nitrogen per gram dry weight of the material. The total nitrogen of roots and shoots was adjusted to the dry weight of the bark.

Results

It was found in early tests that the total nitrogen per gram dry weight of the bark was greater than that of the wood, and that the

TABLE I

TOTAL NITROGEN ANALYSES OF CUTTINGS SHOWN IN
FIGS. 1 AND 2

PART USED FOR NITROGEN DETERMINATION	NORMAL MG. N PER GM. DRY WEIGHT		INVERTED MG. N PER GM. DRY WEIGHT	
	Bark	Wood	Bark	Wood
Morphological apex..	15.0502	2.8330	15.7058	2.7309
Morphological base..	14.9859	2.7791	15.6129	2.6508

proportion of bark to wood became greater from the base to the apex, so that it was necessary to determine the wood and bark separately. The roots and shoots were also determined separately. In showing the gradient of total nitrogen, the total nitrogen of the roots and the shoots was added to the total nitrogen of the bark. This incurred a slight error, for the nitrogen of the shoots and the roots did not come solely from the bark, but a small amount from the wood. The error was small, however, so that it did not greatly affect the value of the results.

Table I shows the distribution of total nitrogen from cuttings shown in figs. 1 and 2. The total nitrogen analyses were made 8 days after the experiment was in progress. There were no visible signs of shoot or root development in either cutting. In both cuttings (nor-

mal and inverted), the morphological apices show larger amounts of total nitrogen per gram dry weight in both wood and bark than the morphological bases, although the differences are not large. The high
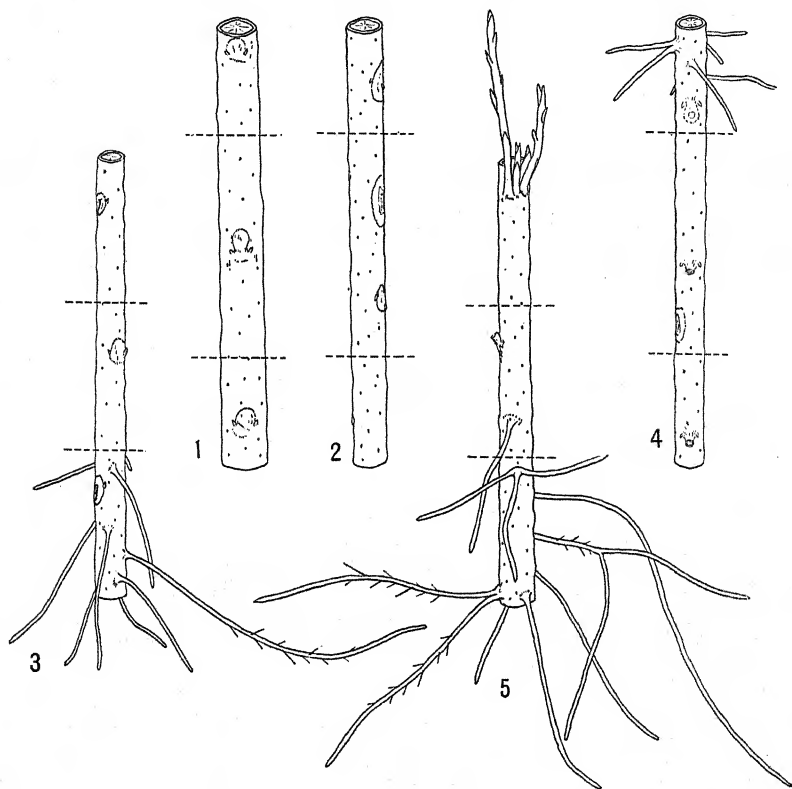
FIGS. 1–5.—Figs. 1, 2, cuttings after 8 days: 1, normal position; 2, inverted position (apical part above and basal part below dotted lines used for analyses). Figs. 3, 4, cuttings after 12 days: 3, normal position; 4, inverted position; both cuttings show strong root polarity at morphological base (apical part above and basal part below dotted lines used for analyses). Fig. 5, cutting in normal position, showing both strong shoot and root polarity (apical part above and basal part below dotted lines used for analyses).

total nitrogen at the morphological apex is due to the normal nitrogen gradient of the cutting. The results indicate that at this early stage, when changes prior to regeneration and development are taking place, there is little if any translocation of nitrogen toward the apex.

Tables II and III show analyses of cuttings shown in figs. 3 and 4, after the experiment had been in progress 12 days. Both cuttings show a strong root polarity at the morphological base. The buds were swelling, although no opening could be observed. The appearance of roots prior to shoot development is not an abnormal condition due to cultural methods, for GOFF (4), JONES (7), and CURTIS (2) found that in a large number of plants under normal conditions, roots appear before the shoots. The results show that when the total nitrogen of the roots is added to that of the bark, the gradient of

TABLE II

TOTAL NITROGEN ANALYSES OF CUTTING SHOWN IN FIG. 3

PART USED FOR NITROGEN DETERMINATION	MG. N PER GM. DRY WEIGHT			
	Bark	Roots	Total	Wood
Morphological apex..	17.6185	....	....	2.5049
Morphological base..	16.9351	0.6060	17.5410	2.4618

TABLE III

TOTAL NITROGEN ANALYSES OF CUTTING SHOWN IN FIG. 4

PART USED FOR NITROGEN DETERMINATION	MG. N PER GM. DRY WEIGHT			
	Bark	Roots	Total	Wood
Morphological apex..	17.4732	....	....	2.7055
Morphological base..	16.9095	0.1284	17.0380	3.6270

total nitrogen is from the morphological apex to the base, regardless of the position of the cutting. Analyses of the wood show that in the cutting with a normal position (fig. 3) the gradient of total nitrogen is also from the morphological apex to the base; but in the inverted cutting (fig. 4) the gradient is from the morphological base to the apex. This shows that at this stage of development in the inverted cutting there is a larger amount of total nitrogen per gram dry weight of the wood at the morphological base than at the morphological apex.

Table IV shows analyses of a cutting (fig. 5) with a well developed root and shoot polarity. The shoots develop in the area of highest total nitrogen per gram dry weight of the cutting, and roots regen-

erate in the area of lowest total nitrogen per gram dry weight of the cutting; although the results show only small differences in the amount of total nitrogen from the apex to the base.

TABLE IV

TOTAL NITROGEN ANALYSES OF CUTTING SHOWN IN FIG. 5

PART USED FOR NITROGEN DETERMINATION	MG. N PER GM. DRY WEIGHT				
	Bark	Shoots	Roots	Total	Wood
Morphological apex......	15.9756	2.4527	....	18.4284	2.5799
Morphological base......	17.2040	....	0.6410	17.8451	2.7022

CURTIS (2) concluded that normally very little, if any, food is translocated up the main stem to be used in shoot growth. DENNY and STANTON (3) have shown that the resumption of growth of the bud of the lilac does not depend upon the remainder of the plant, but is the result of conditions within the bud. The writer's results seem to coincide with those of CURTIS, DENNY, and STANTON, and show that changes prior to regeneration and development in cuttings of *Salix nigra* are not due to a rapid translocation of nitrogen from the base toward the apex.

Summary

1. Willow shoots develop in the area of highest total nitrogen per gram dry weight of the cutting; and roots regenerate in the area of lowest total nitrogen per gram dry weight of the cutting, in cuttings having a normal nitrogen gradient, suspended in a normal position, and with a well developed root and shoot polarity.

2. Analyses of material in the early stages of development seem to indicate that the initial changes prior to regeneration and development are not dependent upon the rapid translocation of nitrogen toward the apex.

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    LOUISVILLE, KY.

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BRIEFER ARTICLES

PREPARATION OF COPY[1]

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A simple, direct statement of facts, shorn of all unnecessary words, should be the contributor's goal for effective writing. It should be borne in mind that the paper is addressed to an audience of trained botanists, already conversant with the fundamentals of the science. Readers do not need to have general botanical terms defined, nor common botanical knowledge repeated. Some contributors, especially those with less experience, resort to much repetition in an effort to make their points clear, instead of presenting the information effectively in the first place. The majority of papers submitted to this journal would be greatly improved in quality by drastic cutting.

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Before the manuscript is typed, a copy of the BOTANICAL GAZETTE should be consulted, and the style followed as closely as possible, especially regarding the arrangement of literature citations, tabular material, and illustrations. Information regarding the maximum length of a paper and the amount of illustrative material accepted may be found on the inside front cover of the journal.

The following specific recommendations are not arbitrary, but are based upon reasonable requirements:

1. Use white paper of the regular typewriting size ($8\frac{1}{2}''\times11''$).

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[2] Footnote.

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12. Include a **summary** in all manuscripts except short ones. A **paragraph of acknowledgments,** if included, should follow the summary.

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> **39.** SATINA, SOPHIA, and BLAKESLEE, A. F., Studies on biochemical differences between $(+)$ and $(-)$ sexes in Mucors. Proc. Nat. Acad. Sci. **11**:528–534. 1925.

Literature citations should be arranged according to the alphabetical sequence of the author's names, and referred to throughout the text by a serial number. If several titles by the same author are included, each should have a separate serial number.

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margins, with black pencil, and indicated as clearly as possible. Since changes in proof may be charged for if numerous, the contributor should refrain from unnecessary revisions. It is recommended that the author read proof through twice: once primarily with the eye, noting each individual word; and once with the ear, reading in phrases for the sense or meaning.

Illustrations

The author must first decide whether his illustrations are to be prepared for use as plates or as text figures. Progress in the engraver's art has been so great during the last decade that excellent results may now be secured with the use of text figures, and this form of illustration has grown in favor with contributors. It has an advantage for the reader, who finds his illustration right at hand when reading the text. There is considerable economy to the publisher also: a full page of line drawings costs $23 if used as a plate; $5.30 if used as a text figure. Contributors are encouraged to prepare illustrations for use as text figures whenever possible.

1. **Consecutive numbering.**—Illustrations, whether used as plates or text figures, are numbered in the order of their reference through the text; that is, the first illustration referred to in a paper should be numbered " 1," the second " 2," and so on. This continuous numerical sequence should obtain even when both plates and text figures are included with the same paper. There should be only one fig. 1, the first illustration on the second plate being numbered to follow the last on the preceding group.

2. **Size of reproduction.**—The available space for text figures is 4 inches in width, and, including the legend, $6\frac{1}{2}$ inches in length. Illustrations for plates should be $5'' \times 7\frac{1}{2}''$ when reduced; that is, the proportion of the width to the length should be as 2 : 3.

METHODS OF REPRODUCTION

With rare exceptions, the BOTANICAL GAZETTE confines itself to two modes of reproduction: zinc-etching and half-tone.

3. **Zinc-etching.**—This method is used for graphs, charts, and all line drawings which do not have extremely fine lines or extremely small dots. The process is less expensive than the half-tone method. Stout drawing paper or Bristol board which is dead white, not creamy or grayish, and jet-black India ink should be used. Pale ink or a wash or tint prevents the use of this process. The effect of a wash can be secured by varying the strength of the lines and the density and size of the stippling. Bold drawing on a scale large enough for half or more reduction will give better results than fine work done under a lens on a scale permitting only slight

or no reduction. Fine dots are not only unnecessary but often disappear in reproduction. Care should be taken to maintain the same scale of strength in all the lines and the same general size in all the printing or lettering.

4. **Half-tone.**—This method is used for the reproduction of photographs and photomicrographs. In making the negative, use a "contrasty" plate, develop with a "contrasty" developer, and print on glossy black-and-white paper. Contrast should be over-emphasized since some of it is lost in reproduction.

MOUNTING

5. **Trimming.**—All unnecessary background should be trimmed before the figure is mounted. Trim away excessive blank paper around the drawings and all except the object to be illustrated in photographs or photomicrographs. Such trimming lessens the amount of reduction required.

6. **Background.**—Pure white cardboard or Bristol board should be used. In all cases the background should be firm enough to support the individual figures without wrinkling. Wrinkles in a photograph tend to show as shadows in reproduction. Library paste is not permanent enough for use in pasting. Use a glue or good mucilage (or Rexo's gummed mounting squares) for drawings, and a rubber cement for photographs (Abbott's rubber cement is recommended).

7. **Grouping.**—Text figures should be mounted into groups whenever possible, using the full page width (4 inches). After the margins are trimmed, the figures should be mounted as closely as possible. Economy of spacing means less reduction in reproduction, a distinct advantage when fine details are to be shown as clearly as possible. Often the full width of the page can be utilized by placing two figures side by side rather than one under the other, and the result is more pleasing.

8. **Index lettering.**—It is most important to use printed letters and figures large enough to reduce with the rest of the illustration and retain their legibility. The smallest size used obviously should be the one considered in this connection. Sheets of printed figures and letters will be supplied by the editorial office upon request. Index letters should be mounted reasonably close to the object they designate.—THE EDITORS.

CURRENT LITERATURE

BOOK REVIEWS
Revision of the Chicago textbook

Recent publication of the revised edition of the Chicago textbook[1] again makes this volume available to students and teachers. The new edition appears in three separate volumes. The morphology text, Volume I, by the late JOHN M. COULTER, remains essentially the same as the earlier edition except for a 5-page bibliography of general literature on thallophytes, bryophytes, pteridophytes, and spermatophytes at the end of the book.

The physiology text, Volume II, has been thoroughly revised by C. A. SHULL. Although the chapter headings and much of the text in the original edition by C. R. BARNES have been retained, the revised edition has been improved by an expansion of more than 100 pages in subject matter, insertion of footnote citations to original literature, addition of a list of standard reference texts at the end of each chapter, and enlargement of the index. The new edition contains amplified discussions of soil moisture, mineral nutrients, osmotic phenomena, the cohesion theory, fat synthesis, enzymes, fermentation, and plant growth and movement.

The new subject matter describes recent advances in plant physiology in the same lucid style found in the original. The bibliographical references constitute a very valuable addition. They will greatly assist inquiring students in finding more information about topics cursorily treated in the text, and will at the same time familiarize them with the current literature of plant physiology. Although citations to foreign literature may not be a great help to beginners, they reveal the international character of scientific activity as well as the value of foreign languages in scientific work.

Although the text has been prepared for undergraduate use, it appears to the reviewer that the subject matter is advanced in character for college students pursuing an introductory course in botany. The extremely concise presentation gives the contents an enormous scope which beginners will find difficult to compass in its entirety; and though SHULL has done a great deal to improve the presentation of difficult subject matter, instructors who use the text in elementary courses will be obliged to simplify and elucidate the description of the more intricate physico-chemical processes. Recent changes in the character of college education and modern developments in the life sciences may also make it desirable for teachers in undergraduate courses to temper the scientific austerity of the book by frequent reference to plant physiological features of human interest.

[1] COULTER, J. M., BARNES, C. R., and COWLES, H. C., A textbook of botany, revised. Vol. I, Morphology. pp. viii+310. *figs. 618.* Vol. II, Physiology. pp. vi+307. *figs. 87.* American Book Company. New York. 1930.

The excellent stylistic composition suffers in only a few places from vagueness and the possibility of misconstruction. Botanists will, for instance, question the wisdom of referring to the cell wall as a membrane (p. 5) when later this term is used in another sense (p. 21). The discussion of imbibition (pp. 6, 7, 9), soil colloids (pp. 48, 50), and autumnal coloration (p. 128) hardly possesses the clarity requisite in an elementary text. Casual mention of suction pressure in a short paragraph at the end of the discussion of turgor and osmotic pressure (pp. 25, 26) might lead to confusion concerning the character and rôle of these forces. The concept of the isoelectric point (p. 48) is introduced abruptly and apparently with little justification as no attempt is made to develop its physiological significance. Mention of the photoelectric cell might have been included in connection with the discussion of the thermodynamics of photosynthesis (p. 228). The foregoing comments concern minutiae and probably express difference of opinion rather than criticism. SHULL has performed an excellent task in bringing this classical textbook of plant physiology up to date.

The Chicago textbook continues to be a masterful presentation of fundamental subject matter. Its terseness, accuracy, and scope will retain for it the preeminent position this text has so long enjoyed. On the whole, it appears that the new edition, like its predecessor, is especially well adapted for use at intermediate levels of instruction. Its appearance in separate volumes admirably adapts it for use in intermediate courses, and this form of the new edition suggests the publishers' recognition of this possibility. The revised edition has gained in excellence of presentation and printing, while the three volumes of smaller size add to its convenience and durability.—W. F. LOEHWING.

Plant physiological chemistry

There has long been needed a book summarizing the chemistry of the metabolic processes of the plant. There are several good plant chemistries on the market, but these are written largely from the chemical rather than the physiological point of view. There has been needed a volume giving the chemistry required for an adequate understanding of the vital processes of the plant, without drawing too largely on the mass information which the pure organic chemists have by their research accumulated in regard to the properties and reactions of the various organic compounds. This need has been adequately met by HARVEY's recent book.[2] In the words of the author, "It is the purpose of this text to present the physiological-chemical mechanism of the vital processes of plants." No attempt is made to give a complete discussion of organic compounds; but only that chemistry is introduced which is thought to be needed to understand the life processes of the plant.

The book is divided into an introduction and six parts. The introduction consists of a discussion of the mechanism involved in the transformations of material. Catalytic action is discussed, and a treatment given of enzymes and their

[2] HARVEY, R. B., Plant physiological chemistry. pp. xix+413. *figs. 119.* Century Company. New York. 1930.

action. Part I consists of a discussion of the general metabolic processes of the plant. Among the subjects treated in this section are absorption, the inorganic nutrients, chemosynthesis, and the nitrogen cycle. Part II is a treatment of the carbohydrates and their uses in the metabolic processes of the plant. Part III discusses the fats, lipides, and waxes; and part IV, the proteins. Parts V and VI are devoted to photosynthesis and respiration. An interesting and valuable feature of the book is the inclusion of photographs of a number of men who have been leaders in the development of plant physiology, most of these being accompanied by quotations from their important publications. Most plant physiologists know all too little about the history of their subject.

Some will think that the author has at certain places been too arbitrary in the choice of a theory to explain a process, and will feel that some indication should have been given of other possible theories; but the author has considered it important to present the theory best fitting the facts, although it may be not without exception and it may be proved false in the future, rather than to confuse the student by presenting too much controversial material.

There is a well selected bibliography at the end of the volume, but the references are not specifically referred to by number in the text of the book. The author tried to avoid too profuse citation of literature, thinking that it confuses the student.

It is a difficult matter to summarize the chemistry of the plant's life processes, and the author has done his task well. All plant physiologists and those in fields using plant physiology as a tool will want at hand a copy of the volume.— S. V. EATON.

Self-sterility and hybrid sterility

Volume 21 of *Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere* is a review of the problems of sterility by BRIEGER.[3] The brief introduction considers sterility as related to ontogeny, the heritability of sterility, and an explanation of the sense in which he uses the term "parasterility," a new term coined to obviate misunderstandings in the discussion of sterility phenomena. The term is used to designate "incompatibility," but in a broader sense than that used by STOUT in 1916.

The main part of the work deals with parasterility among the higher plants, first with reference to flower structure (dichogamy, hercogamy), and then with reference to pollen physiology. The latter section contains a general review of pollen physiology and fertilization, followed by consideration of self-parasterility accompanied by cross-fertility, cross-parasterility accompanied by self-fertility, incomplete parasterility, parasterility from heterostyly, and parasterility in case of species crosses. A brief summary concludes the pollen physiology section.

The last 100 pages of the book take up parasterility in animals, and in thallophytes and protista, with a final chapter of general conclusions. These

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[3] BRIEGER, FRIEDRICH, Selbststerilität und Kreuzungssterilität. 8vo. pp. xii+393. Springer, Berlin. 1930. 33.8 RM, bound.

general conclusions deal with the problems of adaptation, inbreeding degeneration, and sexuality and parasterility.

There are 118 illustrations; and a literature list of over 700 titles, which indicates a thorough summary of the existing literature on sterility. Those interested in this field of biology will find the volume an interesting and valuable guide to a great body of facts.—C. A. SHULL.

Plant dispersal

How plants have traveled to all parts of the globe is a question of perennial interest to students of plant science. Answers to many phases of this universal question have been given by RIDLEY[4] in an encyclopedic volume of over 700 pages. The author has collected observations and records from many sources, as evidenced by the 500 citations of literature on the subject. To this he has added data from his own observations and from unpublished records of other naturalists.

The material has been organized under the main headings of dispersal by wind, by animals, by birds, by reptiles, by simple adhesion, by adhesion through special modifications, by adhesion due to viscid exudation, by human agency, and by mechanical dispersal. Under these headings subdivisions are introduced, based on plant families, genera, and species and also on a taxonomic organization of dispersing birds and insects. The index is one of the plant species concerned, but this together with a table of contents will make the varied contents of the volume readily available for reference. The book should prove a useful guide in a broad but widely explored field.—G. D. FULLER.

Plants of the Gold Coast

This semi-popular description of the plants of the Gold Coast, Africa, has been prepared by IRVINE[5] for use in the region indicated. It is unique in giving the native names of the plants, using for the spelling of these names an alphabet with half a dozen new characters foreign to English books. The major portion of the volume is devoted to non-technical descriptions of native plants designated by their botanical and their native names. The latter are often given in the dialects of four or five different tribes.

The interest of botanists in general will center in the descriptions of the vegetation, which comprises evergreen rain forest, deciduous forest, secondary evergreen forest, savanna forest, savanna, grass savanna, grassland, strand communities, and mangrove associations. There are also lists of plants used for various economic purposes.

The illustrations include some 50 excellent photographs, well reproduced, that serve to convey a vivid picture of the various plant associations.—G. D. FULLER.

4 RIDLEY, H. N., The dispersal of plants throughout the world. pp. xx+744. *pls. 22*. L. Reeve & Co. Ashford, England. 1930.

5 IRVINE, F. R., Plants of the Gold Coast. pp. xxxix+521. *figs. 69*. Oxford University Press. London and New York. 1930. $1.75.

NOTES FOR STUDENTS

The lichen genus Cladonia.—A scholarly taxonomic study by Evans[6] states that 45 species of *Cladonia* are now known in Connecticut, and that many of these are represented by a number of "forms," making a total of 163 in all. An illuminating account of the morphology of *Cladonia* precedes the systematic treatment of the individual species. It is shown that a *Cladonia* plant consists of two portions, the thallus or purely vegetative part homologous with other lichens, and the branched podetium or apothecium-bearing area which arises from the upper surface of the thallus or from the margin of one of the thallus scales. In the subgenus *Cladina*, to which reindeer moss (*Cladonia rangiferina*) belongs, the thallus is small and evanescent, but in the two other subgenera it may be long persistent. Keys to species make use, not only of usual morphological features, but in many cases employ color reactions with potassium hydroxide. Explanations of local distribution of species show attention to ecological details. Since the genus *Cladonia* and many of its species are of worldwide distribution (on all continents, including Australia), this monograph, which expresses the last word in lichen taxonomy, will be of service far beyond the bounds of Connecticut.—F. Ramaley.

Climate and plant species.—Seeds or vegetative specimens from 31 species were collected by Turesson[7] in different climatic regions of Europe and west Asia, and then cultivated together in an experimental garden in southern Sweden. The natural biotypes (forms, varieties) of each of the species were carefully noted and their persistence or modification in the garden critically studied.

Among summer-blooming species, plants brought from the north and plants from the south when grown together tended to retain in large measure their differences in stature, although it is true that the northern plants became somewhat taller and the southern plants showed a certain amount of dwarfing. Yet in the same plot of ground the northern plants were consistently lower-growing than those from the south, and bloomed earlier. These differences are truly genetic, for in any species transplants and seedlings behave in the same manner. Spring-blooming plants when moved north and south to an area of mid-latitude behaved differently as to time of blooming; those from the south bloomed earliest, those from the north came next, while the latest to bloom were the plants native to the region of the experimental garden. Turesson offers an explanation of this phenomenon and discusses his methods of determining the geographic origin of horticultural forms.

The author's work was carried through a number of years, and involved careful handling of material in the garden as well as arduous travel, much of it in wild and forbidding territory. He is to be congratulated for showing beyond peradventure that in many species the mild-climate and severe-climate forms are not mere ecological variants, as might be assumed, but are instead biotypes of different hereditary nature.—F. Ramaley.

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[6] Evans, A. W., Cladoniae of Connecticut. Trans. Conn. Acad. Arts Sci. **30**: 357–510. 1930.

[7] Turesson, Göte, The selective effect of climate upon plant species. Hereditas **14**: 99–152. 1930.

THE

BOTANICAL GAZETTE

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A MICROCHEMICAL AND MORPHOLOGICAL STUDY OF THE DEVELOPING ENDOSPERM OF MAIZE[1]

Lois Lampe

(WITH PLATE III AND SEVEN FIGURES)

Introduction

The endosperm of *Zea mays* L., as of other cereal grains, has been looked upon as a place of food storage with the special function of contributing materials to the embryo during development of the kernel as well as during its germination. No evidence has appeared in this study, however, to support the idea that the embryo brings about any digestion or makes any use whatever of the polysaccharides being stored in the living endosperm cells during the period of kernel development. On the contrary, the two structures develop simultaneously, and the endosperm appears as a competitor of the embryo, sharing the same receptacle and the same food supply. In its development, therefore, it appears to be as distinct an entity as the embryo.

The aim of this paper is to describe the development of the endosperm of the genetic types of corn, starchy, waxy, sweet, and waxy-sweet, in terms of cellular activity. The investigation was begun in 1924 in the botanical laboratories of Ohio State University, and later finished here in 1927. In the interim it was carried on at The Boyce Thompson Institute at Yonkers, New York. No attempt has been

made to separate the findings made at the two institutions. The results reported are from the plantings of corn studied at the Institute in the summer of 1926.

Review of literature

Most of the ideas which have been advanced to explain the phylogenetic significance of the endosperm were early summarized by SARGANT (25). TRELEASE (30) has suggested that the endosperm be called the *xeniophyte*, since it may be regarded as a structure distinctly not forming a part of either the gametophyte or sporophyte generations, and since it first shows the occurrence of crossing where this is recognizable in the developing seed.

The origin of the endosperm of corn by triple fusion was established by GUIGNARD (9) in 1901, and was first illustrated in publications by WEATHERWAX (32) and MILLER (20). GUIGNARD also saw the coenocytic condition of the very young endosperm and the parietal position of the free nuclei in the embryo sac. WEATHERWAX (34) described the centripetal wall formation, which was found to begin when about 50 free nuclei are present and to continue until the endosperm is a complete cellular body. The early development and shape of the tissue were recorded for starchy corn by POINDEXTER (24), whose figures show that the embryo is surrounded at the beginning by the endosperm and that it is laterally placed at the dorsal side of the tissue. FISK (8) noted the poorly restricted cambial activity in the very young endosperm. MOTTIER (22) described the more specific cambial region present in the cap or crown of the kernel in both a sweet and a starchy type of corn during the milk stage in development, and followed the appearance and differentiation of the aleurone layer during the same period. MANGELSDORF (18) reported small starch grains as occurring in the upper cells of the endosperm at the early milk stage, 15–20 days after pollination. By the time the late milk stage was reached, 25–28 days after pollination, he found that the cells in the upper part of the endosperm were completely packed with starch grains, although those in the lower part were still relatively clear. His paper includes microphotographs of sectioned normal kernels at the younger stages mentioned.

That the endosperm of corn has a specific ontogeny has long been

recognized. Calculations from the records of KIESSELBACH (14) for
starchy corn grown during midsummer field conditions in Nebraska
from 1911 to 1921 show an average growth period of approximately
55 days for the whole kernel. APPLEMAN and EATON (1) and CUL-
PEPPER and MAGOON (6) have demonstrated that the rate of growth
of the kernel, and hence the growing period of the endosperm, is
measurably affected by environmental conditions, particularly tem-
perature. LAMPE (15) found that rate of growth of the kernel under
different temperature conditions is expressed in the regional progress
of growth in the endosperm, and in the consequent distribution of
carbohydrates.

Data showing the effect of maturity upon yield are indirectly of
value in determining when polysaccharide storage in the endosperm
actually ceases to occur. As early as 1889, SHELTON (26) measured
the increase in bushels per acre for several starchy varieties of corn,
harvesting at four cardinal points in the development of the kernel.
He showed that corn allowed to mature completely on the stalk
continues to increase in yield for some time after it is usually cut,
the increase being as great as 10–12 per cent after the husks are dry
and the leaves are turning, which was recorded as the usual stage in
Kansas for cutting the crop. Similar measurements were made re-
cently by THATCHER (29) at Wooster, Ohio, where the weekly incre-
ments in acre yield, measured in bushels of shelled corn harvested
and in test weight per bushel, indicate an increase in yield propor-
tional to the degree of maturity attained by the crop.

During the last 75 years many chemical analyses have been made
of the mature kernels. Those of PEARL and BARTLETT (23) on
starchy and sweet corn, and of JONES (12) on starchy, pseudo-
starchy, and sweet types, made to demonstrate the effects of Men-
delian segregation, are representative of the results obtainable.
HOPKINS, SMITH, and EAST (10) carried out detailed chemical analy-
ses of the different parts of mature kernels of starchy corn, and
STRAUGHN (28) a similar but more limited examination of those of
sweet corn.

Analyses mainly of the kernels of sweet varieties at or near the
edible stage have also been made by many workers. CULPEPPER and
MAGOON (5, 6) were the first to make extensive periodic studies of

developing kernels of both sweet and starchy types. Their analyses were carried out on samples taken 5–30 days after pollination, and in the case of later plantings, up to 60 days, when an approximate equivalent of the 30-day kernel was found. The work of these men is of further importance since their analyses show reducing and non-reducing sugars, water-soluble and total polysaccharides. The amount of water-soluble polysaccharide is also evident indirectly in the results of JONES (12).

Studies of (1) the nature of the polysaccharides synthesized in the endosperm of sweet corn, (2) the nature and plastid origin of the units elaborated, and (3) the determining hereditary allelomorphs, were summarized by LAMPE and MEYERS (16) in 1925. Recently BRINK (3) has published data on the chemical properties of the polysaccharide in the endosperm of the waxy type which was originally discovered by WEATHERWAX (33). A bibliography comprising a great variety of papers was assembled by WEATHERWAX (34), and many references to chemical studies have been listed by CULPEPPER and MAGOON (5).

Production of experimental material

In studying the progressive development of the endosperm of corn, it is necessary to begin with healthy vigorous plants which under suitable growing conditions will furnish it with a constant abundant food supply. On the basis of their appearance, the plants used fulfilled these requirements.

CONDITIONS OF GROWTH.—The results reported in the present paper, except where indicated, were obtained from plants grown at the Boyce Thompson Institute at Yonkers, New York, during the summer of 1926. The plots were situated on well drained upland loam soil.[2] The rows were spaced at 3 feet, and the seeds planted 18 inches apart in the row. Two plantings were made from each lot of seed, the first on May 13 and the second on June 8. Cultivation was done by hoeing.

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[2] The soil was described by GUY W. CONREY of the Department of Soils of Ohio State University: as (1) Gloucester loam having a brown color with a grayish cast; (2) situated near the highest point in an upland on the east bank of the Hudson River at an elevation of 320–340 feet above sea level; and (3) nearly level, sloping slightly to the southeast. See soil map, White Plains, New York area, 1919.

SEED.—The starchy type included Burr-Leaming dent and a flint variety. The former was from a double cross of inbred lines of the dent type originally developed by JONES (13) at the Connecticut Agricultural Experiment Station. The latter was an F_2 inbred generation from a cross between inbred lines from two varieties of flint corn. The seed of the waxy type was an F_1 generation from a cross between inbred lines homozygous for the waxy factor. The seed of the sweet type was from reciprocal crosses between two good quality inbred strains of the Golden Bantam variety. The Jones pseudo-starchy seed was of a relatively pure inbred line of sweet corn which was originally selected by JONES (12) for its pseudo-starchy character. The waxy-sweet type was represented by an F_4 inbred generation of a segregate homozygous for the recessive allelomorphs *su wx*, originally obtained from a cross between waxy and ordinary sweet corn. All the seed was from plants grown in 1924 or 1925.

SEASONAL DEVELOPMENT.—The growing season of 1926 at Yonkers was somewhat unusual, in that July and the early part of August were exceedingly warm, dry, and with but few days of cloudy weather. During this period the watering system in the garden was used frequently on the plants. Later, however, there was much rain and consecutively cloudy days.

The progressive development of the plants is of interest in this study only as it affects that of the endosperm, and observations made previous to pollination were not recorded. At pollination certain differences characteristic of the varieties studied were apparent. The Jones pseudo-starchy sweet and Burr-Leaming dent were free from suckers, although the former averaged 4 feet in height and the latter 8.5 feet. Golden Bantam sweet and flint had from one to three suckers, the former averaging 5 feet in height, and the latter 6.5 feet. Waxy and waxy-sweet had four or five suckers to the plant, the former being about 7 feet high and the latter 6 feet. Varietal differences were apparent in the length of the ear shoots. By tests made on the internode of the stalk situated above the ear, and on the shank at the second internode from the base, it was found during the time samples were taken that the stalk and shank in all the varieties were succulent and that the juice was very sweet. The amount and sweetness of the juice were less in the last

three collections of Golden Bantam, which were made 45–60 days after pollination. Even at 60 days, however, a moderate amount of sugar was present, as indicated by taste and by the osazone test.

During growth, the small leaves at the base of the stems of all the plants, which had appeared in the earlier stages of development, progressively died away. The upper leaves were uniformly succulent and green during the period of study, however, except those of Golden Bantam when the last three collections were taken. In this variety a gradual dying back from the tassel was noted, which appeared at the edges of the upper leaves at 40 days after pollination, in all the leaves at 50 days; and at 60 days the uppermost internode of the stalk and all the leaves were dead. The rest of the stalk was alive and greenish, and the leaves and stalk of the one sucker were also moderately succulent.

There was a progressive dying back of the husks in all the plants, which usually reached the middle of the ear at 35–40 days after pollination. The husks of Golden Bantam and flint were respectively dry and yellowish at the time the 50- and 55-day collections were made. An occasional plant became infected with smut and was removed from the garden as soon as noticed.

EVALUATION OF MATERIAL.—The first planting furnished all the collections used from the Jones pseudo-starchy and Golden Bantam sweet corn, and the last collection only, of the flint, waxy, and waxy-sweet types. The second planting was otherwise used as the source of material of the flint, Burr-Leaming dent, waxy, and waxy-sweet types.

Differences in the rates of development of the endosperm in the types studied were not determined for the same set of conditions because the plants did not reach the stage of pollination at the same time, and effects of differing environmental conditions were apparent. The periods of pollination of the plants used from the different types were as follows: flint, August 7–11; Burr-Leaming dent, August 15–17; waxy, August 17–20; Golden Bantam sweet, July 24–28; Jones pseudo-starchy, August 4–11; waxy-sweet, August 17. The last collection, made from the three types flint, waxy, and waxy-sweet, was from ears pollinated earlier, the first on July 28, and the other two on August 11. The number of days per sample between

the pollination and that of collection, therefore, were not equivalent in the amount of growth accomplished. Differences in vigor are also known to occur between inbred and crossbred strains. The general trend in development of the endosperm was nevertheless apparent, and it is to this phase of the problem that the present study is devoted.

Collection and handling of material

AGE OF SAMPLE.—WEATHERWAX (32) found in a variety of sweet corn in early September that about 24 hours elapsed between the time of pollination and that of fertilization. BUCHHOLZ and BLAKESLEE (4) have shown that under the usual conditions of heredity and environment, growth of pollen tubes in *Datura* is nearly uniform. Accordingly the age of the kernels was counted in days from the time of pollination to that of collection, no deduction being made for the time required for pollen tube growth. CULPEPPER and MAGOON (5, 6) had previously calculated the age of the kernels on open-pollinated ears in the same way.

The plants were hand-pollinated because cross-pollination would have occurred, and because kernels of known age were desired. Pollinations were made after the method employed by corn geneticists, so timed that ears filled with kernels to within an inch or two of the tip were obtained. All the ear shoots on a stalk were bagged until the time when the two upper ears were pollinated. The pedigree and the day and hour of pollination were recorded.

SCHEDULE OF SAMPLING.—During the most active part of the growing period, a study of the kernels in each variety was made every fifth day, and near the middle of each 5-day period. As the season advanced one sample every 5 days was taken. At longer intervals the whole plant was brought to the laboratory, when progress in development of the stalk, leaves, and ear shoot was observed. Observations and preparations from samples were usually carried out between 4 o'clock in the afternoon and 11 o'clock in the evening. The four earliest stages of Golden Bantam sweet corn, however, which were taken in the middle of the day, and three morning collections from other varieties, showed no significant difference in results because of the time of collection.

PROCEDURE IN SAMPLING.—Samples were taken at the time the

observations and preparations were to be made, the record sheets
and reagents having been previously prepared. Only the upper ear
of a plant was studied. One-half of the ear was collected at a time,
and placed in a labeled bag. The husks and ear-bag were replaced
over the other half of the ear. It had been shown by APPLEMAN and
EATON (1) that the rate of development of the remaining kernels is
not measurably affected by the removal of part of the ear if the
husks are closed and held in place. The results of the writer during
the previous season were in agreement, but the lower half of the ear
was collected only when necessary. Two samples were usually taken
at one time, the second being kept in a moist chamber in the ice box
while the first was being handled. The kernels nearest the middle of
the ear were studied when the upper half of it was collected, although
the outer 1 inch was discarded when the lower half was used.

The order of observations, preparations, and recording was uni-
form for all samples, and was governed by the rate at which specific
changes were likely to occur in the material. The record of the taste
of the stalk and shank when these were tested, and the preparations
for reducing and total sugars in the kernel were always taken care of
first. The rest of the study followed in the order found to be most
efficient. The procedure for one sample required less than 1 hour.
Moisture samples from the late afternoon collections were afterwards
prepared late at night, and those from the evening collections the
next morning. During the interim the portions of the ears furnishing
these samples were kept in a moist chamber in the ice box. There was
no discernible change during this period in the kernels used.

Microchemical methods

In the chemical study it was not intended to find additional proofs
for substances long accepted as present in the endosperm of corn,
nor to search for those unidentified or reported only in traces. The
aim was to find the localization of substances present in abundance,
by a few reliable tests, for the bearing that the results would have
upon the interpretation of endosperm development.

CUTTING AND WASHING SECTIONS.—Freehand sections cut longi-
tudinally through the center of the kernel and the embryo were used.
A small cavity, apparently resulting from tissue tensions, was usual-

ly found in the center of the older endosperms, and was therefore often present in the sections (fig. 11 c). For plastid and polysaccharide study the sections were about two cells thick, and for the sugar tests about 0.5 mm. thick. The depth of the latter provided many uncut cells and accentuated the localization of the resulting crystals. All sections were washed by twirling them rapidly in a Syracuse watch glass half-filled with distilled water. Forty revolutions requiring about 15 seconds were found to be satisfactory. The water was poured off immediately and the sections drained on clean filter paper. They were then transferred to the reagent desired. Washing was necessary since plastids and sap came freely out of the large cut cells, and good localization of materials was otherwise impossible. Even then critical observations were always made on uncut cells.

IODINE REAGENT.—An iodine solution, consisting of 0.3 gm. of iodine and 1.5 gm. of potassium iodide in 200 cc. of distilled water, was used in the study of plastid development and the consequent distribution of polysaccharides. Tests identifying the substances stored by the plastids as carbohydrates had been made earlier by LAMPE (16), and for the sake of speed in observing, the iodine reagent alone was used. The dilute solution neither distorted nor disintegrated the cell contents during the period of observation, yet the polysaccharide was sufficiently stained. By its use the desiccated globules were shown to be still intact in mature sweet corn, a fact not suspected at the publication of the preliminary paper (16), when the stronger reagent was being used.

A solution of twice the strength just noted was employed in making gross observations on sections and cut kernels, because of the more intense coloration produced. It was particularly useful in finding the extent of the core of globule-containing cells in sweet corn. This reagent was found to darken the protein content of the endosperm, and after some time to cause swelling of the grains of carbohydrate, especially in the younger kernels, and to disintegrate the compound grains and globules in the sweet varieties. The water medium and the pressure of the cover glass at the drying down of the mount also appeared to be factors in disintegration of the compound grains. When the current observations were completed, sections of

the kernels stained with iodine were remounted in levulose syrup (5 parts levulose; 3 parts water) and stored. The stain gradually faded out, although distribution of the solid carbohydrate was still evident.

POLARISCOPE.—This instrument had been used earlier (16) in the study of the bodies elaborated by the plastids. It was found also to be of value in the study of the decreasing moisture content of the kernel, as the grains of starch and waxy starch did not show the characteristic cross in the dark field until their moisture content was lowered either artificially or in the natural process of kernel maturation.

REDUCING AND NON-REDUCING SUBSTANCES IN KERNEL.— WEHMER (35) has listed glucose or invert sugar, saccharose, dextrin, and starch as having been reported in quantity in the corn kernel, and mentions tannin and pentosans, all of which have reducing power, either before or after acid inversion. VER HULST (31) reported 0.18 of 1 per cent free pentoses and 6.4 per cent pentosans in the kernels of starchy corn at the dent stage. Because only the reducing sugars, sucrose, dextrin, and starch are considered in the great bulk of quantitative work reported by other workers, and because of the specificity of the tests employed, these substances will be mentioned in the interpretation of the tests used in the present study.

INVERSION OF SUCROSE.—Invertase prepared in purified liquid form by Dr. H. R. KRAYBILL was used for inversion of the sucrose. It gave very satisfactory results when used with the phenylhydrazine reagent. The sections of the kernels were mounted in the undiluted invertase solution and placed on a warm plate kept at 45° C. until the excess moisture evaporated. While they were still moist the phenylhydrazine reagent and cover glasses were added.

OSAZONE TEST FOR REDUCING SUGARS.—A solution was made from two preparations: (1) phenylhydrazine hydrochloride in glycerin (1:10) and (2) sodium acetate in glycerin (1:10), which were mixed in the proportion of 1:2 at the time of using. Ten median sections from kernels on different sides of the ear were selected and the embryos removed. The remains of the kernels and the embryos were assembled separately in two rows on a 2×3 inch slide, and grouped conveniently for covering. The reagent was added directly to the

sections in the upper row, and after treatment with invertase to the
lower. When the mounts were completed the slide was put on the
warm plate (45° C.) and left for approximately 20 hours. The use of
low temperatures in the study of sugars has been recommended by
IRVINE (11), and the time of heating was found to be satisfactory in
the plan of work followed. At the end of this period the rosettes of
large crystals characteristic of the osazones of reducing sugars were
striking. The crystals were well localized, the upper row of sections
indicating the amount of reducing sugars present, and the lower the
amount of total sugars, and hence, sucrose by difference. All the
slides were examined and later placed in slide boxes which were
stored in an upright position to prevent slipping of the mounts.
MANGHAM (19) has noted that such preparations keep indefinitely
when out of contact with air. The mounts prepared by the writer,
although unsealed, showed no change in amount of precipitate pres-
ent during the 8 months the slides were under frequent observation.
Even now, 3 years later, the amount and distribution of crystalliza-
tion are apparently unaltered, although fresh reagent has been added
a few times to replace that lost by evaporation.

COPPER-REDUCTION TEST FOR REDUCING SUGARS.—Fluckiger's re-
agent, a sky-blue solution of copper tartrate powder in 20 per cent
sodium hydroxide, which was made up at the time of using, was
employed to a slight extent. The interpretation of this test is known
to be governed (1) by the reducing substance present, (2) by the
relative amounts of sample and reagent mixed together, (3) by the
temperature of the mount, and (4) by the time of standing previous
to observation. Uniformly small amounts of the pure sugars were
tested separately. Fructose appeared to produce the maximum
amount of cuprous oxide precipitate in 7 minutes at room tempera-
ture, and glucose, in 3 minutes at 45° C. Using commercial dextrin,
the precipitate obtained in 5–20 minutes at 45° C. was considered
to be a measure of the reducing power of the dextrin present. In the
study of corn, however, the amount of sugar in the younger kernels
was so great that satisfactory localization was not possible with this
reagent. It also formed a white gel with the invertase solution and
its use as a general test was abandoned.

SENSITIVITY OF SUGAR TESTS.—SIMON (27) states that Fehling's

solution, a copper-reduction test for reducing sugars, is sensitive to 0.0008 of 1 per cent, and that the osazone test is sensitive to 0.5–0.025 of 1 per cent. The relative sensitivity of the two types of reagents is shown in fig. 10 *b*, where the results of five tests are shown, three with Fluckiger's reagent (which is similar in reaction to Fehling's solution) and two with the phenylhydrazine reagent. One of the last two mounts was inverted. Before inversion the endosperm gave no crystals with the phenylhydrazine reagent, and only a small

WAXY CORN

a,
— HORNY REGION WITH ANGULAR GRAINS } WAXY STARCH
— FLOURY REGION WITH SPHERICAL GRAINS } (NO JUICE)
— ZONE OF MATURATION
— PLASTIDS AT NUCLEI: IMMATURE CELLS WITH MILKY JUICE
— NO PLASTIDS AT NUCLEI: IMMATURE CELLS WITH WATERY JUICE

38 DAY

b,
— DISTRIBUTION OF OSAZONE CRYSTALS AFTER INVERSION
— DISTRIBUTION OF CUPROUS OXIDE CRYSTALS AFTER 20 MIN. AT 45°C., AND REGION CONTAINING CRYSTALS AT END OF:—
— 20 MIN. AT 45°C.
— 3 MIN. AT 45°C.
— 7 MIN. AT ROOM TEMPERATURE

TMM   4

FIG. 10.—Longitudinal sections of kernels of waxy corn 38 days after pollination, showing: *a*, position of gross internal regions of kernel and regional progress in cellular development and maturation; *b*, results of five tests for reducing and non-reducing substances. Amount and distribution of reducing substances indicated in endosperm, and that of non-reducing substance (sucrose) in embryo; minute amount of cuprous oxide precipitate obtained in hypocotyl of embryo not represented.

amount of precipitate with Fluckiger's in the basal part. The results obtained may be accounted for on the basis of the sensitivity of the two reagents, and indicate the presence of a small amount of reducing substance. The scarcity of non-reducing substance is apparent since the osazone test made after inversion again produced no crystals.[3] Previous to inversion the embryo gave negative results with the phenylhydrazine reagent, and only a minute amount of precipitate with Fluckiger's, in the region of the hypocotyl. The results

[3] The ear furnishing these kernels was collected in the late afternoon, March 20, 1926, from a plant growing in the greenhouse. It was examined as to general external

indicate the virtual absence of reducing substances. An abundance of non-reducing substance was present, however, since the osazone test made after inversion gave a heavy precipitate.

Because the amount of reducing substances other than the hexose sugars has not been found to be significant in the kernels of this species, and because there were no indications of a lack of penetration nor any interference with the reaction of the phenylhydrazine reagent or of the invertase, positive tests have been used to indicate the presence of reducing sugars, and negative tests to indicate their absence. The lower sensitivity of this reagent should be kept in mind, however, when the average distribution of sugars based on results with it is made.

PREPARATION OF MOISTURE SAMPLES.—The procedure employed by plant chemists was followed, except that a definite number of whole kernels was used. Each was freed from glumes and peduncle, and placed uninjured in the weighing bottle, the lid being lifted each time. The preliminary drying was done at 58° C. in an electric oven and the final drying at 100° C. in a vacuum oven. From these samples the percentages of moisture and dry matter in the kernels were obtained.

EVALUATION OF METHODS.—The microchemical methods recommended by ECKERSON (7) were modified with her help to suit the material used. The results agreed with the quantitative data obtained macrochemically by other workers, and an understanding of the development of the endosperm was made more nearly possible by fitting together the data made available by the two methods. Individual differences in samples and in localization of materials, which are usually covered up in macrochemical analysis by averages of determinations made on large samples, were detected microchemically.

Results from both methods have been of value in this study, in that those obtained macrochemically furnish a basis for quantitative estimation of the ones arrived at microchemically. On the other

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and internal appearance, then stored in a moist chamber in the ice box. The kernels were examined microscopically and for sugars on the following afternoon. The delay was probably responsible for the unusually low amount of sugars present in the base of the endosperm.

hand, the data obtained microchemically interpret and build on to those resulting from macrochemical analysis. The percentage of moisture in the kernels studied makes possible the comparison of results obtained in the present investigation with the quantitative data recorded by other workers. The book of methods of the Association of Official Agricultural Chemists (2) was used in checking the relationships apparent in the results obtained microchemically and macrochemically.

Results[4]

1. ONTOGENY OF AMYLIFEROUS CELL IN ENDOSPERM.—The following description applies only to the cells composing the major portion of the endosperm, not to those of the aleurone layer, nor to the peripheral glandlike cells situated in the basal portion of the endosperm above the vascular plate.

Starting with a very young cell in the endosperm, there was always a certain amount of enlargement, not only of the cell as a whole but of its nucleus. Development of vacuoles and increase in cell size were most prominent in those cells situated in the central portion of the endosperm. The cell walls remained thin and apparently undifferentiated. In general the nucleus was suspended in the vacuole by strands of granular cytoplasm, which showed streaming in the younger cells.

In the early studies (16) it became apparent that the larger granules in the cytoplasm developed into plastids which were responsible for the storage of polysaccharides. A definite grouping of the plastids about the nuclei was found to occur shortly before and at the beginning of storage. At this time differentiation in the plastids characteristic of the four types of corn (starchy, waxy, sweet, and waxy-sweet) was evident. The timing of events in the early growth of a

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[4] A synopsis of the results recorded in this paper was presented before the Physiological Section of the Botanical Society of America, Philadelphia, December, 1927.

At the completion of this manuscript a paper appeared which also deals with the development of the endosperm of corn (WEATHERWAX, PAUL, The endosperm of *Zea* and *Coix*. Amer. Jour. Bot. 17:371–380. 1930). The two papers agree on the following points: (1) the food supply of the older endosperm enters through its base; (2) there is an orderly development of the endosperm and a relatively high degree of differentiation of parts. WEATHERWAX also confirms the earlier report of the writer (15) as to the regional deposition of polysaccharides in the developing endosperm.

cell in the endosperm, as well as the changes which occur in it later, are given below. The rate of development described is that characteristic of healthy plants growing under the midsummer field conditions of 1926, at the Boyce Thompson Institute, Yonkers, New York.

The larger cells in the central part of an endosperm 5 days after pollination contained granules scattered throughout the cytoplasm, many of which were protoplastids (fig. 1). In the more advanced cells of a 7–10-day endosperm the developing plastids were grouped about the nuclei (figs. 2, 3). At about the tenth day, polysaccharide storage began in the cells in the upper central part of the endosperm (fig. 3). By about the twelfth day, the plastids in the more advanced cells were no longer grouped about the nuclei but were scattered within the cytoplasm (fig. 4).

As the plastids increased in size, the cytoplasmic network in which they were imbedded practically filled the cell, and the vacuole was relatively reduced and no longer obvious. In so far as noted, the plastid population of an individual cell developed uniformly and at about the same time. A small amount of variation in the size of the grains occurred during the period of development, however, and afterwards in the mature kernel.

A study of plastid development was made the previous season, using waxy corn pollinated August 15, 1925. Measurements of the grains of polysaccharide in the most advanced cells of the endosperm were made daily. They increased steadily in size from $1.5\ \mu$ at 10.5 days after pollination, when polysaccharide storage in the endosperm began, to $18.2\ \mu$ at 30 days. Subsequently no significant increase in their size could be detected, and further discernible changes in the cells occurred only at the drying down of the kernel. When the maximum size of the grains of carbohydrates was reached, they were closely packed and angular in the region of the endosperm which later became horny; they were loosely packed and nearly spherical in the region which matured floury. The distribution of grains of the two shapes is shown in the fig. 10 a, which, however, represents a kernel after drying down had begun.

2. ONTOGENY OF ENDOSPERM.—What was hitherto known of the development of the endosperm is in the main summarized in the

review of literature, and a sufficient presentation of the initiation of the endosperm may be found there. Collections in the present study began 5 days after pollination.

The very young endosperm, 5–8 days after pollination, was made up of cells much alike in appearance, except (as noted by FISK [8]) that the peripheral ones were smaller and less vacuolate. The lowest peripheral cells lying above the vascular plate began early to have more dense protoplasm and somewhat thickened walls. The gland-like appearance of these cells was maintained throughout the development of the tissue.[5]

Polysaccharide storage began about 10 days after pollination. It occurred first in the upper central part of the young endosperm, and spread gradually to neighboring cells. The region soon extended diagonally toward the periphery at the dorsal side of the endosperm above the embryo. By 12–15 days after pollination, a cap-shaped zone of polysaccharide-storing cells thus appeared below the base of the silk, which spread outward in all directions as more and more cells reached an advanced stage in development. There was a gradation in degree of cellular maturity, extending from this zone of cells in the cap of the endosperm which showed maximum polysaccharide storage, to those at the base of the tissue which were least advanced in development and not yet storing polysaccharides. At the same time there was a similar range in cellular development, although much shorter, extending from the zone of more mature cells in the cap outward to the periphery. At about 18 days after pollination the zone had increased in extent until it occupied the whole upper central portion of the endosperm. In the 20-day kernel one or more layers of immature cells, which were free from polysaccharides, were still present beneath the aleurone layer in the cap, but at 25 days they had usually disappeared through the initiation of polysaccharide storage, from a small area at the center of the periphery of the cap of the endosperm. By 30 days the area had increased, occupying the major portion of the upper cap. As development pro-

[5] L. F. RANDOLPH described the glandlike cells in connection with a paper (Embryogeny and endosperm formation in maize) presented before the General Section of the Botanical Society of America, New York, December, 1928. WEATHERWAX (see footnote 4) considered these cells to have a placental function with respect to the entrance of food materials into the endosperm.

ceeded it gradually spread around the sides of the cap and downward over the base of the tissue.

Always there was a transition band of cells which separated the cells showing maximum polysaccharide storage from those in which it had not begun. In this band there was a complete series of cells in the early stages of plastid development. Figs. 5 and 6 show two cells of the transition band in the lower central part of an endosperm at approximately 15 days after pollination. The first had small plastids which were collecting about the nucleus and not yet storing polysaccharide. The second cell, situated in the endosperm above the first, had larger plastids which were grouped about the nucleus, and in which polysaccharide storage had begun.

Since the transition band represented a gradation in the progress of cellular development, it was migratory and early progressed outward to the periphery of the cap, and more gradually downward into the base until the peripheral tissue was reached. Its position in a maturing kernel of waxy corn is shown in the stippled region of fig. 10 a. Two consecutive stages in its downward progress in maturing sweet and flint corn are shown in figs. 14 a, b, and 15 a, b. The few basal cells in a nearly mature endosperm (fig. 14 c) may not store any polysaccharide. No grains of carbohydrate were found in the peripheral glandlike cells bordering the vascular plate.

The position and duration of the regions of growth in the endosperm were indicated by (1) the conformation of cells, (2) the progressive enlargement of cells, (3) the degree attained by the plastids of successive cells in the synthesis of polysaccharides, and (4) the progressive maturation of cells. While meristematic activity seemed upon these bases to be everywhere present in the periphery of the tissue, it appeared to be of longer duration in the basal half of the endosperm. Final proof as to whether the continued juvenile appearance of the cells in this region is due to delayed development or to later origin rests upon the demonstration of division figures in the cells. But whichever interpretation proves to be correct, their progressive development after plastid activity began was continuous and uniform.

In this report a cell in the endosperm is designated as immature when its plastids have accomplished little or no synthesis of poly-

saccharides; it is considered mature when carbohydrate storage by the plastids appears to be complete. Growth is indicated by plastid activity in the cells, and maturation by the changes occurring at the end of the growth period, both in a cell and in the endosperm as a whole.

3. EFFECT OF PRIMARY HEREDITARY FACTORS DETERMINING ENDO-SPERM TYPES UPON POLYSACCHARIDE STORAGE.—While the ontogeny of the amyliferous cells in the endosperm was found to be uniform in the different types of corn studied, the end point reached in the synthesis of polysaccharides and the visible appearance of the units elaborated were affected by the genetic complex of the kernel. To insure clarity, the concept as to the expression of the determining hereditary factors in the endosperm and the results of their segregation (summarized in the main by LAMPE and MEYERS 16) will be briefly reviewed.

In the non-sweet types, starchy and waxy, there are moderately large simple grains of carbohydrate. These grains in the starchy type are composed of starch. In the waxy type they are composed of a closely related substance, which has been called erythrodextrin by WEATHERWAX (33), and waxy starch by BRINK (3). The grains of starch stain blue with iodine solution, those of the waxy starch stain red. In the sweet types, ordinary sweet and waxy-sweet, there are compound grains of carbohydrate and also globules of liquid dextrin which may or may not contain small grains, similar in nature to the small grains composing the larger compound ones. The grains of carbohydrate in sweet corn stain blue with iodine but in waxy-sweet corn they stain red. The liquid content of the globules stains red with iodine. Regardless of form, size, position, and reaction with iodine, all the grains of carbohydrate when dehydrated and of sufficient size are bright, and show the dark cross in polarized light which is characteristic of starch. The liquid content of the globules fails to show the dark cross, being dark throughout.

The compound grains of carbohydrate characteristic of the sweet types of corn, originally illustrated in immature kernels of Golden Bantam by MOTTIER (22), are shown in fig. 7. The globules also found in sweet corn, many of which have tiny grains within them, are shown in fig. 8. The storage units characteristic of the non-

sweet types (photographed earlier by JONES 12, and BRINK 3) are represented in fig. 9.

The kind of carbohydrate and the form of the storage unit produced are determined largely by the interaction of the two pairs of hereditary allelomorphs Su su and Wx wx, established by geneticists as in the main responsible for the differences in development of the endosperm in the four genetic types of corn. The presence of the dominant factor (Su) is responsible for the development of the large simple grains of carbohydrate. The presence of the recessive factor (su) and the absence of Su result in the development of globules and compound grains in the sweet types, regardless of whether the dominant Wx or the recessive wx is present. The allelomorphs (Wx, wx) influence the development of the grains of carbohydrate, so that when the dominant Wx is present starch is synthesized, and when only the recessive wx is present, waxy starch is synthesized.

4. EFFECT OF CELL POSITION UPON POLYSACCHARIDE STORAGE.— It is well known that the peripheral cells of the mature endosperm of all types of corn and the units of carbohydrate which they contain are always smaller than those in the central portion. In waxy corn regional variation in plastid activity, aside from the size of the unit elaborated, was also found to occur. The cells in the central region of the cap of the kernel contained grains of waxy starch, staining red with iodine solution throughout. In cells situated progressively farther away from this central region, the red-staining grains of carbohydrate had increasingly larger center-spots of blue. At the base of the endosperm, and sometimes beneath the aleurone layer, small grains entirely blue-staining were found. These little grains, as well as the center spots in the larger ones, which were shown up after fading of the mounts had occurred, lost the blue iodine stain upon heating and regained it upon cooling, just as starch does, and are considered to be of starchy character. The immature cells of the maturing waxy kernel, whose location is indicated in the stippled region of fig. 10 a, were elaborating the small blue-staining grains mentioned. A similar reaction was found to be characteristic of the grains of carbohydrate in the basal cells and often in other peripheral cells of the endosperm in waxy-sweet corn.

Regional variation in plastid activity was also evident in the

endosperm of sweet corn. In the sweet and waxy-sweet types the globules in the central region of the cap often contained no granules of solid carbohydrate at all. In a zone situated at some distance away in every direction, the globules usually contained small solid granules which developed within them. These granules were characteristically of starch in sweet corn, and of waxy starch in the waxy-sweet type. In cells situated still farther outward, the plastids produced globules which contained larger and larger granules, both simple and compound. The plastids in the peripheral cells of the varieties reported characteristically gave rise only to compound grains.

The change in plastid activity from the elaboration of globules to the development of grains was definite, so that a pattern was produced which was easily visible in longitudinal sections of the kernels. The peripheral zone of cells containing compound grains, and designated as the starchy region in the diagrams of fig. 14, was much more prominent at the base of the endosperm than in the cap. The last few cells to mature sometimes produced small simple grains as in non-sweet corn. In two cases grains a few times compound were found to develop in the non-sweet types.

It appears then that the time of origin of a cell and its position in the endosperm affect the end point reached in the synthesis of polysaccharides. In the presence of the dominant factors Su and Wx, together with the rest of the heredity present, the plastids synthesized starch. The substitution of the recessive su or wx resulted in incompletion of or difference in the process of synthesis, so that the liquid dextrin or the waxy starch was produced. The change in the end point reached in the synthesis of polysaccharides in the waxy and sweet types was more nearly complete in the central region of the cap, where the cells matured early in the development of the tissue. It was least evident in the peripheral cells, particularly in those at the base, where the cells matured progressively later after those in the central region of the cap had reached maturity.

5. EFFECT OF MODIFYING HEREDITARY FACTORS UPON EXPRESSION OF GENETIC FACTOR (su) IN ENDOSPERM OF SWEET CORN.[6]—JONES (12) segregated out by inbreeding and selection a semi-starchy type

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[6] This concept was originally presented in a paper (The mode of expression of factors influencing carbohydrate storage in the endosperm of corn) by LOIS LAMPE and MARION T. MEYERS before the Joint Genetic Sections of the American Society of Zoologists and the Botanical Society of America, Washington, December, 1924.

of sweet corn which he called pseudo-starchy. He concluded from its behavior in breeding experiments that the modified character of the kernels is not due to imperfect segregation, or to contamination of the genetic factor (*su*), but to the sorting out and rearrangement of other hereditary factors affecting its expression. JONES analyzed mature kernels of the starchy, pseudo-starchy, and sweet types macrochemically, and separated out the water-soluble carbohydrates, which would include the sugars present. The fraction from starchy corn yielded 4.45 per cent dextrose; that from pseudo-starchy 7.19 per cent; and that from sweet corn 26.15 per cent. Since the percentage of water-soluble carbohydrate in the fraction from starchy corn is approximately that obtainable as total sugar from mature kernels, the water-soluble polysaccharide appears to be absent in this type. After correction for sugars is estimated in the percentages given for the other two types, it is seen that there is less water-soluble polysaccharide in pseudo-starchy than in good quality sweet corn. The corresponding amounts of water-insoluble carbohydrates calculated as starch from a second fraction were respectively 58.73 per cent in starchy corn, 51.50 per cent in pseudo-starchy, and 29.00 per cent in sweet corn.

The idea advanced by LAMPE and MEYERS (16), that the water-soluble polysaccharide is furnished by the globule content of the cells in the sweet types, explains JONES' analyses. In good quality Golden Bantam sweet corn the number of globule-containing cells was large when compared with the number of cells containing only compound grains, while in the pseudo-starchy type, judging from an extensive examination of material of the JONES' strain, the proportion of cells containing globules to those containing compound grains was small. Some variation in the size of the globule-producing region also occurred in the kernels on the same ear and from plant to plant. Both storage units were absent in starchy corn where only the large simple grains were found. The extent of the core of globule-containing cells in immature kernels of Golden Bantam sweet and Jones pseudo-starchy varieties and its absence in starchy corn are shown in fig. 11. Thus in the endosperm of good quality sweet corn, the influence of the primary genetic factor (*su*) in the presence of the rest

of the heredity results in the development of compound grains and globules in about equal proportions. On the other hand, in the pseudo-starchy type the modifying hereditary factors present affect the expression of the primary genetic factor (su), so as to result in a

FLINT (STARCHY) CORN
—REGION PRODUCING STARCH GRAINS

—IMMATURE TISSUE: NO POLYSACCHARIDE

20 DAY

JONES PSEUDO-STARCHY CORN
—REGION PRODUCING STARCH GRAINS
—REGION PRODUCING GLOBULES OF DEXTRIN

—IMMATURE TISSUE: NO POLYSACCHARIDE

20 DAY

GOLDEN BANTAM SWEET CORN
—REGION PRODUCING STARCH GRAINS

—REGION PRODUCING GLOBULES OF DEXTRIN

—IMMATURE TISSUE: NO POLYSACCHARIDE

20 DAY   1MM.
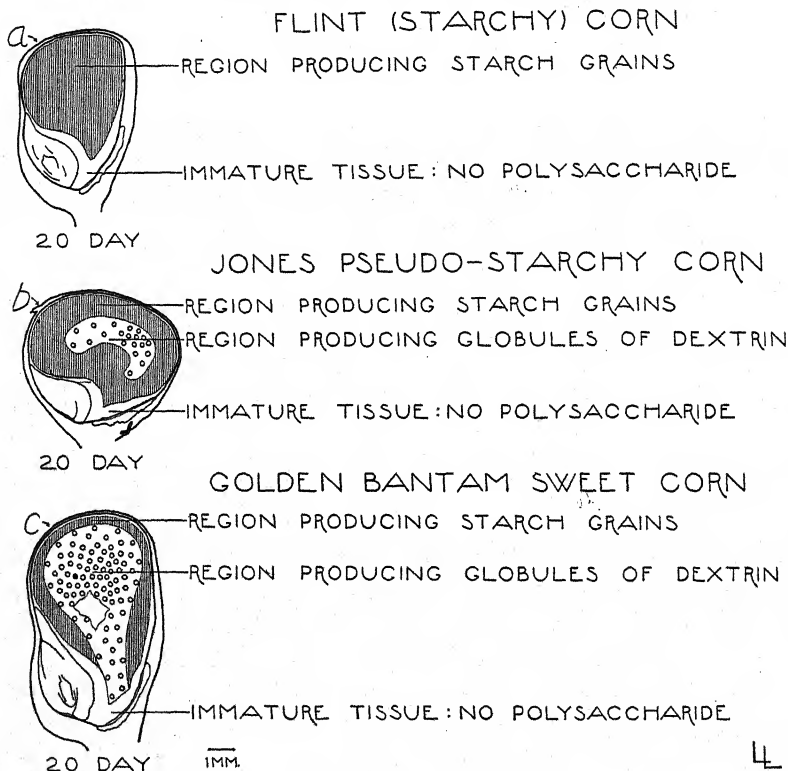
FIG. 11.—Longitudinal sections of kernels of starchy, Jones pseudostarchy, and sweet corn 20 days after pollination, showing regions of kernel whose cells give rise respectively to moderately large starch grains and globules of liquid dextrin which may or may not contain small grains; cavity often present in center of older endosperm shown in c.

decrease in the number of cells producing globules and an increase in the number producing only compound grains. These data indicate that other hereditary factors rearranged by segregation, and considered by JONES to be responsible for the modified character of the endosperm of the pseudo-starchy type, affect development of

the endosperm through their effect on plastid activity in the synthesis of polysaccharides.

It may be seen, however, that the factor for sweet endosperm is still operating even if no globules are produced, because of the abundant compound grains determined by it. Extreme variation in the pseudo-starchy type of sweet corn, leading to the exclusive production of the large simple grains of polysaccharide characteristic of the non-sweet types instead of the globules and compound grains, has not been found.

6. Effect of regional progress of growth upon localization of carbohydrates in developing endosperm.

Polysaccharides.—As described in section 2, the cells in the endosperm first reaching maturity formed a zone in the cap of the endosperm which early spread outward to the periphery and more gradually downward toward the base by the addition of other cells attaining a like stage in development. Since cellular maturity was accompanied by equivalent progress in plastic activity, there was an attendant accumulation of polysaccharides in these cells. The extent of the region affected was thus at any time a measure of the amount and distribution of these substances. Figs. 10, 11, 14, and 15 show various stages in the development of the endosperm and the progress made in polysaccharide storage.

With the exception of the sugars in the embryo, the occurrence and distribution of carbohydrates in the kernel outside of the endosperm was not studied in detail. It was noted, however, that starch was not present in the body of the ovule, and that it diminished during development of the kernel in the pericarp and embryo. It was usually absent from the mature pericarp. The distribution of starch and sugars in the pericarp and that of starch in the embryo are not recorded in the illustrations.

Reducing sugars.—Reducing sugars were present during the early development of the kernel in all the types of corn, where they were found in moderate quantities in the pericarp, ovule, and young endosperm. At this time the path of translocation seemed to be in part through the succulent outer tissues. After the disappearance of the body of the ovule, and the stretching and differentiation of the cells in the upper pericarp, reducing sugars were not abundant in the ker-

nel outside of the endosperm, and appeared to enter the endosperm through the vascular plate, a disk-shaped complex of vascular tissue at the base of the pericarp.

At successive stages in the development of the endosperm, reducing sugars were confined to the immature cells where they were present in uniformly moderate amounts. The relative stage in the development of the endosperm was apparent, not only in the extent of the distribution of polysaccharides, but also in the accompanying regional distribution of reducing sugars. In older kernels the polysaccharides occurred in the more mature cells in the cap, and the reducing sugars in the immature cells at its base. As the region containing polysaccharides increased during development of the tissue, the region containing reducing sugars decreased (figs. 12 *A*, 13 *A*).

Total sugars (sucrose by difference).—Sucrose was not abundant in the endosperm until 10–12 days after pollination. It increased rapidly and reached a maximum throughout the tissue approximately 15 days after pollination in the several varieties studied. As the cap cells gradually matured the amount of sucrose in them decreased. Sucrose was not depleted in these cells as rapidly as the reducing sugars, however, and restriction of the region of cells containing sucrose lagged both in time and extent behind the diminishing region containing reducing sugar. During the entire growth period total sugars continued to be abundant in the basal portion of the tissue. The region indicated gradually decreased in extent and the sugar disappeared at the maturity of the kernel. Figs. 12 *B* and 13 *B* show the distribution of total sugars in the endosperm and embryo of the developing kernels of sweet and starchy corn. In each of the figures comparison may be made of the diagrams in series *A*, which show the distribution of reducing sugars in the endosperm and their absence in the embryo, with the diagrams in series *B* showing total sugars in these structures. The approximate amount and distribution of sucrose, the non-reducing sugar present, may thus be obtained. Total sugars in an older kernel of waxy corn are shown in fig. 10 *b*, where depletion of sugars was accentuated by a period of storage before the tests were made.

Amount and distribution of total sugars in different endosperm types.—While the maximum amount of total sugars reached early in

the development of the endosperm varied little in the different varieties, the amount subsequently present at any one time was specific
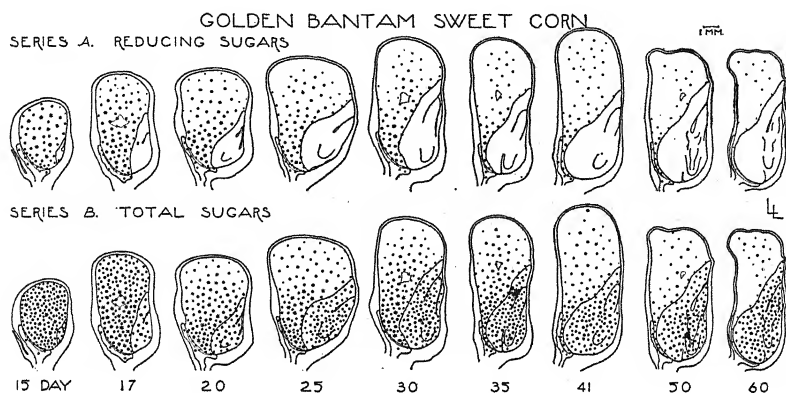
FIG. 12.—Longitudinal sections of kernels of Golden Bantam sweet corn at successive stages during growth period; amount and distribution of sugars as indicated by osazone test both before and after inversion with invertase shown by stippling: A, reducing sugars; B, total sugars; sucrose indicated by difference.
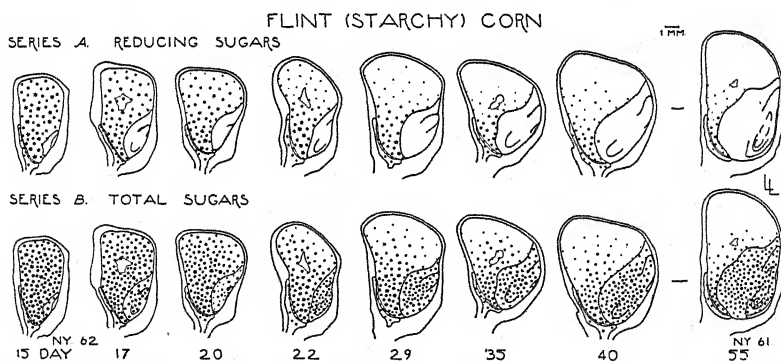
FIG. 13.—Longitudinal sections of kernels of flint corn at successive stages during growth period; amount and distribution of sugars as indicated by osazone test both before and after inversion with invertase shown by stippling: A, reducing sugars; B, total sugars; sucrose indicated by difference.

for the types of corn studied. Owing particularly to the lower amount of sucrose present, such samples of developing non-sweet corn, both starchy and waxy, and of the JONES pseudo-starchy variety, appeared regularly to contain less total sugars than com-

parable stages of good quality sweet corn, both sweet and waxy-sweet. The relative abundance of sugars in the sweet and non-sweet types is represented in figs. 12 and 13.

Differences in the types were also apparent in the amount of total sugars present in the mature endosperm. In non-sweet corn the disappearance of sugars was essentially complete at maturity. The condition is indicated in fig. 13, which shows successive stages in the development of flint corn, and in fig. 10 b, showing a maturing kernel of waxy corn. Sugar was present in the base of the endosperm at all stages represented, however, since no completely matured kernel is shown.

In the sweet varieties, and to a much less extent in JONES pseudo-starchy, a small amount of sugar (mostly sucrose) remained in the mature endosperm, being essentially confined to the central region where the dextrin of globule origin was localized. The small amount and distribution of total sugars in maturing kernels of Golden Bantam sweet corn are indicated in the older kernels of fig. 12 B. Reducing sugars which were even more restricted are shown in the maturing kernels of this variety in fig. 12 A and fig. 14.

Comparison of results obtained by microchemical and macrochemical methods.—The results obtained microchemically in the present study are consistent with the quantitative data obtained by CULPEPPER and MAGOON (5) in their periodic study of sweet and starchy varieties during the first 30 days after pollination. Both kinds of data show that there is a gradual reduction in the amount of reducing sugars, and that there is a rise and fall in the amount of sucrose. The microchemical results explain the quantitative data, however, in as much as they show (1) that there is not a general uniform decrease in reducing sugars per cell throughout the endosperm, but rather a restriction in the number of cells containing them; and (2) that there is an increase and subsequent decrease in the amount of sucrose per cell which is followed by a restriction in the number of cells in which sucrose is abundant.

The quantitative data obtained by STRAUGHN (28) from analyses of different parts of the mature kernel of sweet corn are also in agreement with the microchemical observations as to the amount and distribution of sugars present. Comparison of the 60-day kernel in

fig. 12 with the results of STRAUGHN, indicates (1) that sucrose and no reducing sugar occurs in the embryo, (2) that the amount of sucrose present in the endosperm is greater than that of the reducing sugars, and (3) that the amount of total sugars present in the mature endosperm is greater in the cap than in the base.

The analyses of PEARL and BARTLETT (23), made on mature kernels of starchy corn, agree with the microchemical results in indicating a complete disappearance of reducing sugars from the kernels during maturation. No dextrose was found by these investigators in nine determinations on six different samples, and an average of 0.12 of 1 per cent is obtainable from three determinations on two others. The same samples yielded respectively 1.83 and 1.71 average percentages of sucrose. The microchemical method showed that the sucrose reported in the macrochemical analysis was in the embryo. Maturing kernels are shown in figs. 10 *b*, 13, and 15, although no completely matured kernel is illustrated.

7. REGIONAL CHANGES IN SAP CONTENT OF MATURING ENDOSPERM. —In the foregoing sections of the paper, it was shown: (1) that the ontogeny of an amyliferous cell in the endosperm resulted in abundant polysaccharide storage, and that maturing of the cell was accompanied by a relative reduction in the amount of cell sap; (2) that regional progress in cellular development occurred first in the cap, and spread gradually to the base of the tissue during later stages of development; (3) that a transition band of cells showing all degrees of maturity occurred between the regions of maturing and immature tissue; (4) that this transition band migrated downward to the base of the older endosperm as cells successively entered upon the period of polysaccharide storage; (5) that polysaccharide storage in the cells of this band marked the beginning of the decrease in the relative amount of sap present in the cells.

As a result of the further decrease of sap content during the drying down of the kernel, a second transition band appeared in the endosperm above the one just mentioned. This new zone was narrow, and separated the cells in the cap of the kernel, which contained little or no sap and were completely mature, from those below it, which still contained abundant sap and were immature. Since final maturation and rapid loss of the remaining cell sap appeared to occur in this

zone of cells, it was called the zone of maturation. The line of demar-
cation was definite and associated with the external appearance of
the kernel known as glazing.

The maturation zone was migratory, as was the transition band of
less mature cells just mentioned. Its lowering from the center of the
periphery of the cap to the base of the tissue is called in this paper
the fall of the zone of maturation. The lowering of this zone was
more rapid when drying down first began, because the cells in the
cap were everywhere nearly mature and free from sap. Later its
lowering was less rapid, because the cells at the base of the endo-
sperm approached maturity and complete polysaccharide storage
successively, at the same time lessening the reserve of immature sap-
filled cells.

The presence and fall of the zone of maturation were found to be
characteristic of the endosperm of all the types and varieties of corn
studied. In these experiments the fall of this zone in waxy corn had
progressed one-third of the way down the endosperm at 35 days after
pollination, and at 40 days, in the flint, Golden Bantam sweet, and
Jones pseudo-starchy varieties. It had not begun to fall at 35 days in
Burr-Leaming dent and waxy-sweet corn.

Successive positions of the zone of maturation in the maturing
endosperm of Golden Bantam sweet corn are shown in fig. 14 *a, b*.
It was no longer apparent in the endosperm shown in *c*. Quantitative
data as to the amount of dry matter and the moisture content of the
kernels are also included in fig. 14. From these it is evident that there
was a decrease in the percentage of moisture and a corresponding in-
crease in the percentage of dry matter in progressively older kernels.

When the endosperm was separated from the embryo and divided
at the line of cutting indicated (fig. 14), the moisture content in all
three samples was found to be greater in the base of the endosperm
than in the cap. When the percentages of moisture in the caps and
in the bases of the successive samples are compared, it is apparent
that the loss of moisture in the base was greater during the matura-
tion period than the loss in the more nearly mature cap. When the
kernel was practically mature (fig. 14 *c*) the percentage of moisture
was nearly uniform throughout.

The position of the zone of maturation in maturing kernels of the

non-sweet varieties is shown in figs. 10 *a* and 15. When kernels of flint corn were prepared as were those of Golden Bantam just described, the amount of moisture was again found to be greater in the base than in the cap at the two stages studied. The loss of moisture during the early part of the maturation period here studied was also
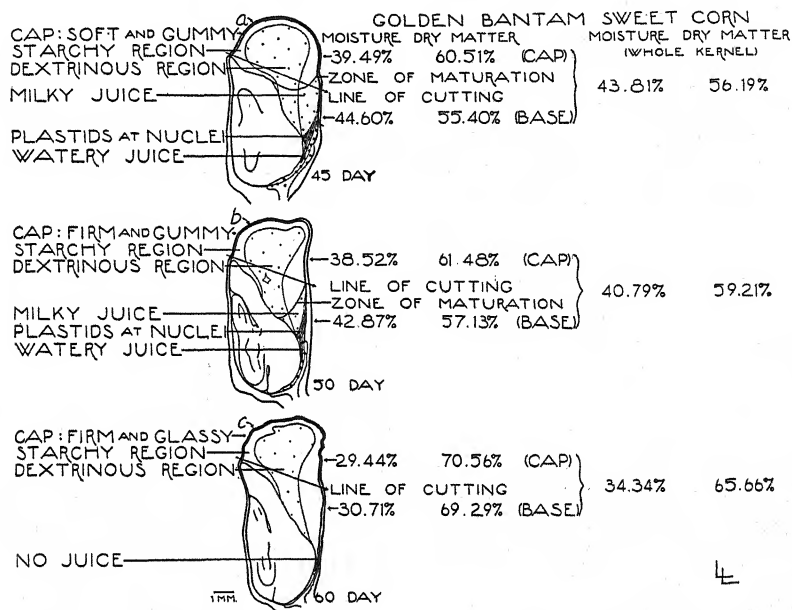
FIG. 14.—Longitudinal sections of kernels of Golden Bantam sweet corn made during later stages of development, showing (1) regional progress in cellular development and maturation; (2) position of gross internal regions of kernel and zone of maturation; (3) moisture and dry matter content of cap and base of endosperm when separated at line of cutting indicated; and (4) moisture and dry matter content of whole kernel. Amount and distribution of reducing sugars shown by stippling.

greater in the base, where the cells were progressively arriving at maximum polysaccharide storage (fig. 15). Greater differences were found in this type than in Golden Bantam sweet corn, for which three factors were primarily responsible: (1) the samples were from two successive plantings; (2) there was a greater difference in the number of days between pollination and collection for the samples used; and (3) the chemical nature of the polysaccharides synthesized was specific for the two types of corn. The changes in moisture con-

tent during maturation of the kernels show the same regional development of the endosperm as is indicated by the regional progress in the maturing of the cells, and by the consequent localization of sugars and polysaccharides.

The characteristics of the gross internal regions of the endosperm which are present during the maturation period determine the gross external appearance of the kernel subsequent to premature or normal drying down of the kernel.
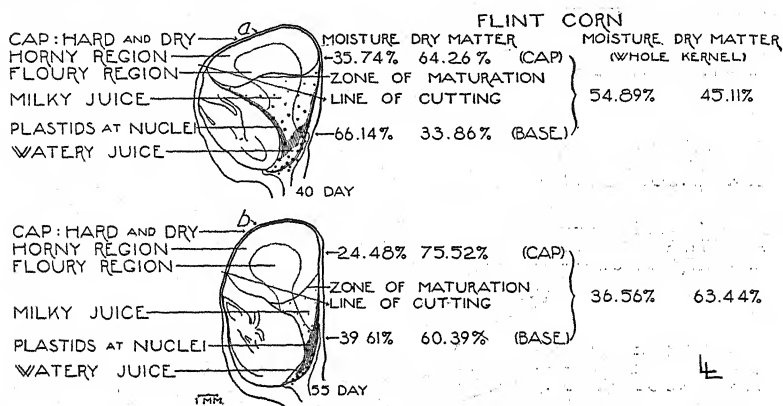
FIG. 15.—Longitudinal sections of kernels of flint corn made during later stages in development, showing (1) regional progress in cellular development and maturation; (2) position of gross internal regions of kernel and zone of maturation; (3) moisture and dry matter content of cap and base of endosperm when separated at line of cutting indicated; and (4) moisture and dry matter content of whole kernel. Amount and distribution of reducing sugars shown by stippling.

Kernels of flint corn 40 days after pollination had succulent tissue at the base of the endosperm, and hard dryish tissue in the cap where the cells were well packed with starch. The base of the endosperm was found to shrink upon drying in proportion to the size of the region affected, and to the degree of succulence present. The cap, however, remained plump and rounded because the cells were well filled with starch, and little moisture was present which could be lost. In the practical judging of corn, kernels shrunken at the base have long been penalized. MOORE (21) pointed out that shrunken kernels are indicative of immaturity, and that the shrinkage results in spaces at the cob, between the bases of the kernels. LYON and MONTGOMERY

(**17**) state that such spaces indicate immaturity, poor feeding value, and a decreased percentage of grain. On the other hand, kernels of flint corn in which the storage of starch had been practically completed in the basal cells of the endosperm, and which lacked succulent tissue here, did not materially change their shape upon drying. A high starch content and low percentage of moisture throughout the kernel resulted in maintenance of the original form. MOORE (**21**) recorded that in well matured corn no noticeable spaces are found at the cob between the bases of the kernels, and that they fit tightly together at the base as well as at the cap. Effects similar to those described for flint corn were apparent also in the kernels of the other types studied, where changes in the cap due to degree of packing or to kind of carbohydrate present complicated in certain ones the results obtained. The gross internal regions of the maturing endosperm of waxy, sweet, and flint corn are indicated respectively in figs. 10 *a*, 14, and 15.

8. PERCENTAGES OF MOISTURE AND DRY MATTER IN DEVELOPING KERNEL.—CULPEPPER and MAGOON (**5**) have already shown that a progressive decrease in moisture content and a like increase in dry matter occur during the earlier stages in the development of the corn kernel. They also found an increase in polysaccharide content which parallelled closely the increase in amount of dry matter. The percentages of moisture calculated for the samples are shown in the curves of fig. 16. Microscopical study of the kernels indicated that decrease in the moisture content of the kernel as a whole began at initiation of polysaccharide storage in the cells of the endosperm, and continued regularly until the drying down of the kernel occurred. Corresponding increase in the percentage of dry matter paralleled somewhat closely the successive maturing of the cells, as indicated by their progress in polysaccharide storage. Thus the percentages of moisture and dry matter in the kernels constitute a fair index to the maturity of the endosperm. The percentage of moisture in the kernel, however, could be an absolute index only provided the embryo and pericarp showed the same changes in moisture content.

The data from the samples of the different types which were of the same age in days after pollination are not entirely comparable, mainly because of the differences in the times of pollination. The

samples from Golden Bantam sweet, flint, and Jones pseudo-starchy varieties, which were pollinated earlier, had a lower average percentage of moisture; and those of Burr-Leaming dent and waxy-sweet, which were pollinated later, had a higher average percentage
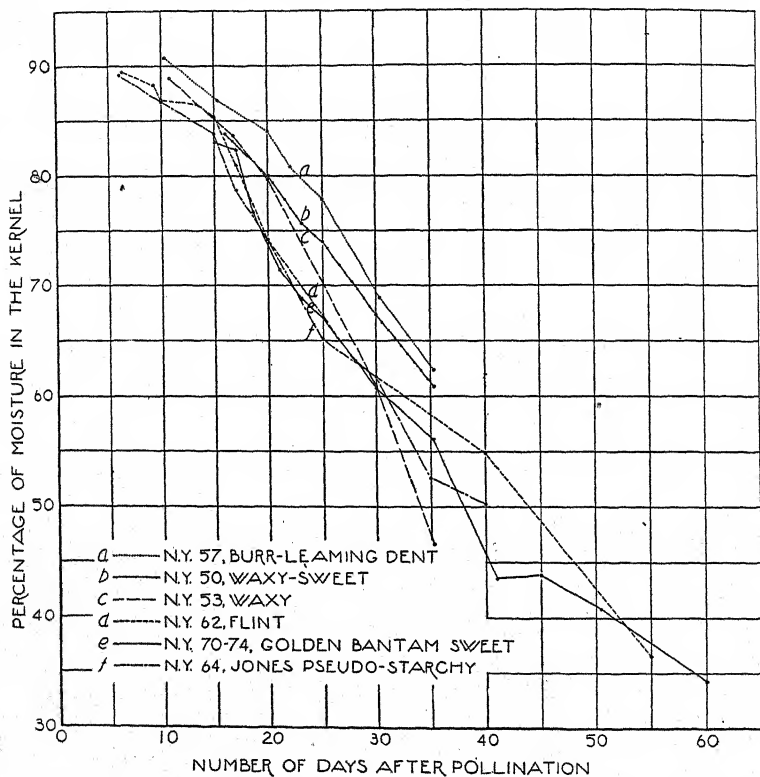
FIG. 16.—Curves showing moisture content of kernels at successive stages of development in the six varieties of corn studied.

of moisture. Hence the curves of fig. 16 fall into two groups. The curve of the waxy type, however, passed from the later to the earlier group. This variety also appeared to mature more rapidly than the two late-pollinated ones.

Discussion

Development of the endosperm in the types of maize studied was characterized by morphological and physiological gradients which

are undoubtedly associated with polarity and symmetry in growth. Regional gradients in cellular development and consequent distribution of materials were evident in the growing endosperm. Such gradients may be assembled into two groups. The members of the one were shorter, and extended inward from the periphery of the endosperm at the cap of the kernel where the meristematic aspect of cells first disappeared. Those of the other group, which continued with decreasing prominence throughout the period of development, extended upward toward the cap from the base of the endosperm where the cells, owing either to arrested development or to more recent origin, retained a juvenile appearance for a much longer time.

Since the ontogeny of a cell ended in abundant polysaccharide storage, there were gradients in these materials which extended from an ever widening region in the upper part of the endosperm outward toward the periphery and downward into the base. After maturing of the cells in the cap, gradients in the amount of materials present also ceased to exist there. Progressively shorter gradients in reducing sugars, sucrose, and moisture content extended from the base upward toward the cap. They virtually disappeared at kernel maturity.

There were also gradients in the character of the polysaccharides synthesized in the waxy and sweet types which extended outward in all directions from the central region in the cap of the endosperm. It was suggested in section 4 that the time of appearance of a cell, and consequently its position with reference to the periphery of the endosperm, affected its plastid activity and the end point in polysaccharide synthesis. Development of every cell appeared thus to be influenced by the presence of those which arose before it, and perhaps to some extent by those which came afterward, and correlations were indicated in the development of the different parts.

The economic importance of the investigation lies primarily in the recognition of the regional maturing of the endosperm in relation to yield and quality of grain at harvest. The continued cellular activity in the base of the tissue explains to a great extent why THATCHER (29) and SHELTON (26) found an increase in yield proportional to lateness in time of harvesting, and also why kernels properly developed and matured are well filled at the base and of maximum dry

weight, as required in the judging of corn. Recognition of the regional maturing of the endosperm has an important bearing also upon the time of collection of sweet corn for best edible quality.

Summary

Progressive development of the endosperm in the four genetic types of maize (starchy, waxy, sweet, and waxy-sweet) was studied by microchemical and microscopical methods.

1. ONTOGENY OF AMYLIFEROUS CELL.

a. Appearance of an amyliferous cell was followed by enlargement of the whole cell, its vacuole, and nucleus. The size attained by the cells of an older endosperm was greater in the central region of the tissue than at the periphery.

b. The plastids developed from granules, the protoplastids, which were present in the cytoplasm. In the more advanced cells, 7–12 days after pollination, the plastids were grouped about the nucleus. At about 10 days, polysaccharide storage began in the more advanced cells, and differentiation in the plastids of the four genetic types was evident.

c. The plastid population of a single cell appeared to develop uniformly and concurrently. The plastids were imbedded in the cytoplasmic network which finally filled the cell.

d. The relative amount of vacuolar sap in a cell decreased during its development, and the vacuole ceased to be observable.

e. The grains of polysaccharide elaborated by the plastids in waxy corn increased in size up to 30 days after pollination. At this time those in the cells of the region which later became horny were angular, and fitted closely together; those in the region which later became floury were loosely packed, and spherical. No further significant change in the cell and its contents occurred until the drying down of the kernel.

2. ONTOGENY OF ENDOSPERM.

a. Peripheral meristematic activity appeared to be everywhere present early in the development of endosperm tissue, and to be of longer duration in the base than in the cap.

b. Polysaccharide storage began first in the upper central part of the endosperm tissue.

c. Regional progress in the development of cells early resulted in a cap-shaped zone of cells, advanced in polysaccharide storage, which was situated in the upper part of the endosperm below the base of the silk. This zone of cells increased in size, spreading outward to the periphery of the cap, and much more gradually downward into the base of the endosperm by the addition of other cells attaining a like stage in development.

d. There was a transition band of cells in which polysaccharide storage was just beginning, which separated the cells showing maximum polysaccharide storage from those in which it had not begun. The band was migratory, and early progressed outward to the periphery in the cap and more gradually downward into the base of the tissue.

3. EFFECT OF PRIMARY HEREDITARY FACTORS DETERMINING ENDOSPERM TYPES UPON POLYSACCHARIDE STORAGE.

a. The end point reached in polysaccharide synthesis in the endosperm and the appearance of the units elaborated are determined in the main by the hereditary allelomorphs Su, su and Wx, wx.

b. In the endosperm of non-sweet corn, presence of the dominant factor (Su) results in the development of rather large simple grains of carbohydrate.

c. In the endosperm of sweet corn, presence of the recessive factor (su) and absence of Su results in development of compound grains of carbohydrate and globules of liquid dextrin. The latter may or may not contain small grains.

d. The allelomorphic pair (Wx, wx) affects development of the grains of carbohydrate. When Wx is present, starch is synthesized as in the endosperm of starchy and ordinary sweet corn. When wx is present and Wx absent, waxy starch is synthesized as in the endosperm of waxy and waxy-sweet corn.

4. EFFECT OF POSITION OF CELL UPON POLYSACCHARIDE STORAGE.

a. In waxy corn the grains of waxy starch situated progressively farther away from the central region of the cap had increasingly larger centers of starchy character. Grains entirely of starch were often produced in the peripheral cells, particularly in those at the base of the tissue. The latter were also found located similarly in waxy-sweet corn.

b. In good quality sweet types the cells in the upper central region contained only globules of liquid dextrin. At some distance outward in every direction, the plastids produced globules which contained progressively larger granules of solid carbohydrate. The plastids in the peripheral cells usually produced only compound grains. This change was definite and easily visible in longitudinal sections of the kernels.

5. EFFECT OF MODIFYING HEREDITARY FACTORS UPON EXPRESSION OF PRIMARY GENETIC FACTOR (*su*) IN ENDOSPERM OF SWEET CORN.

In the endosperm of the pseudo-starchy type the modifying hereditary factors present affected the expression of the primary genetic factor (*su*), resulting in a decrease in the number of cells producing globules, and an increase in the number producing only compound grains. The central core of the globule-producing cells of the Jones pseudo-starchy variety was therefore greatly reduced when compared with that in good quality sweet corn.

6. EFFECT OF REGIONAL PROGRESS OF GROWTH UPON LOCALIZATION OF CARBOHYDRATES IN DEVELOPING ENDOSPERM.

a. Polysaccharides accumulated first in the more advanced cells in the cap, and the region extended progressively outward and downward as the maturing of cells proceeded.

b. Reducing sugars decreased in the cap as the cells approached maturity. They were present in uniformly moderate amounts, in successively fewer immature cells in the basal half of the endosperm.

c. The sucrose content of the endosperm in the several varieties rose gradually to its maximum amount and distribution in the tissue at about 15 days after pollination, and then decreased. The sucrose content of the cells was not lowered as rapidly as that of reducing sugars, however, and restriction of the region containing sucrose lagged both in time and extent behind the diminishing region containing reducing sugars.

d. The amount of total sugar in the endosperm subsequent to about 15 days after pollination was greater in good quality sweet corn than in the Jones pseudo-starchy and non-sweet types.

e. In non-sweet corn disappearance of sugars from the endosperm was essentially complete at maturity. In the sweet types, and to a

less extent in the Jones pseudo-starchy variety, a small amount of sugar (mostly sucrose) remained in the mature endosperm, being practically confined to the central region where the dextrin of globule origin was localized.

f. Data obtained by the quantitative chemical analysis of corn kernels are supplemented by the results arrived at microchemically, since localization of materials in the kernel is thereby made available.

7. REGIONAL CHANGES IN SAP CONTENT OF MATURING ENDOSPERM.

a. At the drying down of the kernel the cells successively and rapidly lost their remaining sap and entered upon the period of maturation. The narrow zone of cells in which this change was occurring was called the zone of maturation. It separated the cells in the cap of the older endosperm which had matured and were practically free of sap from the immature sap-containing cells in the base.

b. The zone of maturation migrated downward as development proceeded. Its lowering from the center of the periphery to the base of the tissue was termed the fall of the zone of maturation. This fall usually began in the kernel 35–40 days after pollination. At the beginning the fall was more rapid because the cells were nearly mature and free from sap. Later it was less rapid because the cells at the base of the endosperm approached maturity and complete polysaccharide storage successively.

8. PERCENTAGES OF MOISTURE AND DRY MATTER IN DEVELOPING KERNEL.

a. The percentage of moisture steadily decreased as development of the kernel proceeded.

b. Increase in dry matter paralleled closely the progress made in the synthesis of polysaccharides as the cells of the endosperm successively attained complete polysaccharide storage.

Gradients in the development of cells and in the attendant localization and distribution of materials, such as the carbohydrate and moisture content, suggest that polarity and symmetry underlie the harmonious development of the endosperm. Correlation between the position of the cell and its plastid activity is indicated by the regional

variation in the nature of the polysaccharides synthesized in the waxy and sweet types.

The economic importance of the investigation lies in the recognition of the regional maturing of the endosperm in relation to time of selection of sweet corn for best edible quality, and in relation to maximum yield of grain at harvest time.

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EXPLANATION OF PLATE III

Amyliferous cells and polysaccharide bodies resulting from plastid activity in endosperm of corn. Figs. 1–6 inclusive are from free-hand sections of fresh tissue mounted in a mixture of 5 per cent sugar solution and weak iodine potassium ·iodide solution; figs. 7–9 are from smears using tissue in the cap of the endosperm and very dilute iodine solution. Magnification in figs. 2–4 inclusive is slightly less than that in the other figures ($\times$0.87).

· FIG. 1.—Golden Bantam sweet corn approximately 5 days after pollination; protoplastids scattered throughout cytoplasm.

FIG. 2.—Waxy corn 9 days after pollination; plastids collecting about nucleus.

FIG. 3.—Waxy corn 11 days after pollination; plastids mostly grouped about nucleus, larger, and beginning to store polysaccharides.

FIG. 4.—Waxy corn 17 days after pollination; plastids scattered about within ·cytoplasm mainly at one side of cell.

FIG. 5.—Golden Bantam sweet corn approximately 15 days after pollination; cell in basal half of endosperm showing plastids collecting about nucleus; some plasmolysis of cell apparent.

FIG. 6.—Same; cell above that shown in fig. 5; plastids showing starch synthesis grouped about nucleus; structure of plastids not shown.

FIG. 7.—Black Mexican sweet corn 50 days after pollination; compound grains of starch from cells in endosperm above tip of embryo.

FIG. 8.—Same; globules of liquid dextrin, many of which contain starch grains; from cells at center of cap of endosperm.

FIG. 9.—Burr-Leaming dent corn 50 days after pollination; simple grains of starch from horny and floury regions in central part of endosperm cap.

PLATE III

1

2

3

4

5

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8

$50\,\mu.$

9

4
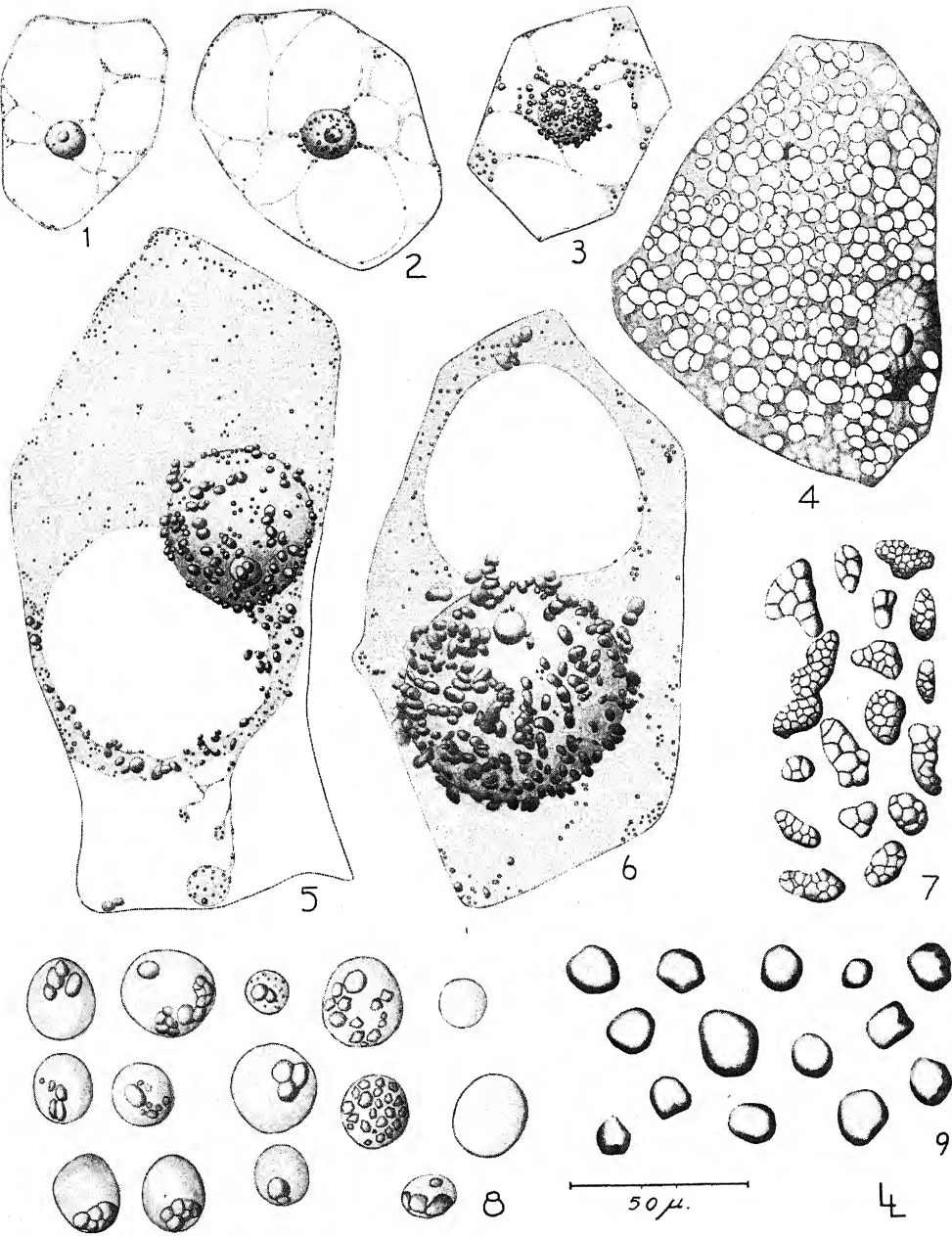
LAMPE on MAIZE
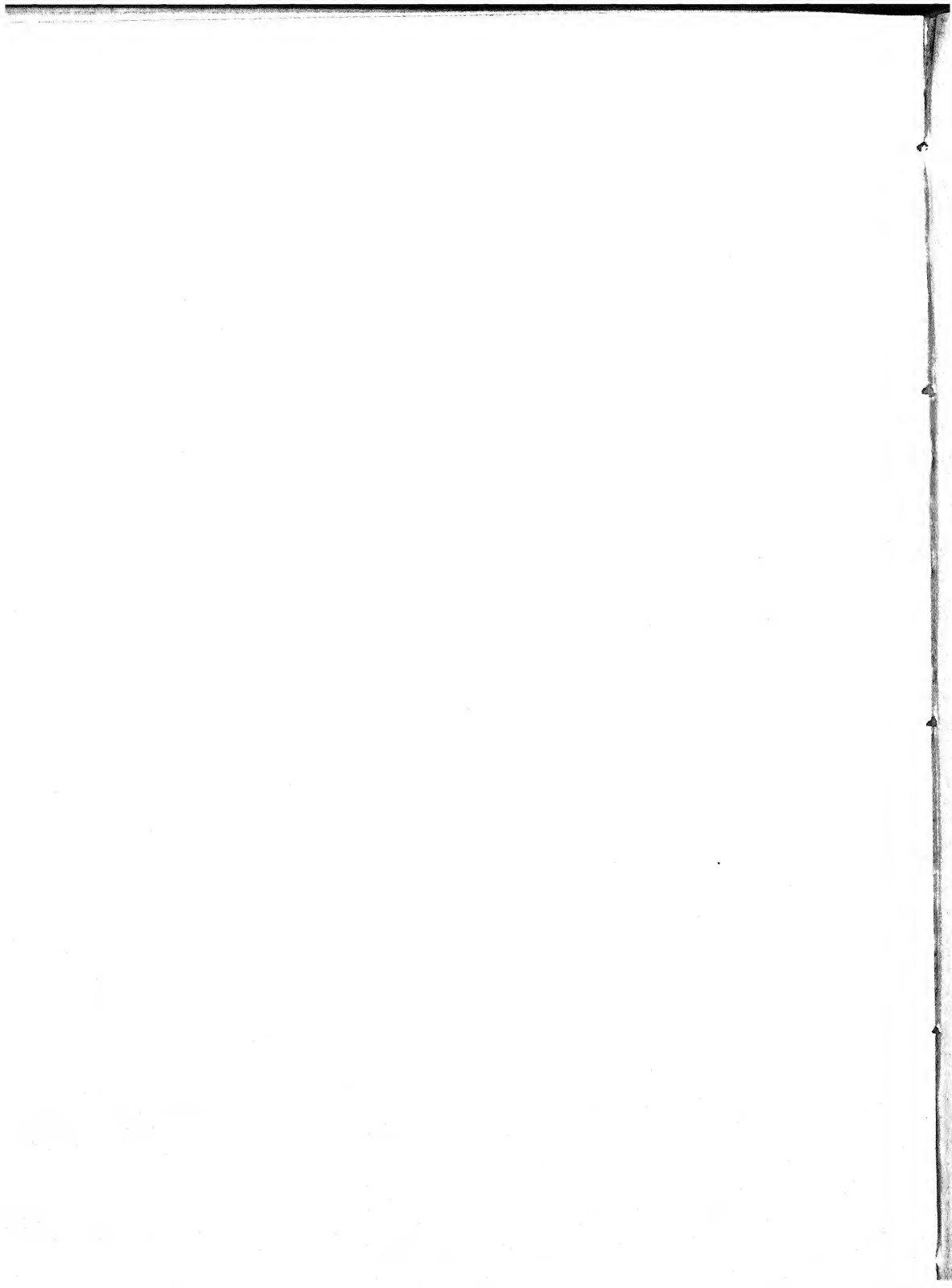
DEVELOPMENT OF DIONAEA MUSCIPULA

II. GERMINATION OF SEED AND DEVELOPMENT OF SEEDLING TO MATURITY[1]

CORNELIA MARSCHALL SMITH

(WITH THIRTY-SIX FIGURES)

Preparation of soil

The seeds of *Dionaea* are difficult to germinate. Of the various conditions under which the writer attempted to grow them, the following proved to be the most successful. The bottom of a porous clay flower pot, 5 inches in height and 6 inches in diameter, was covered with pieces of broken pottery, on top of which was placed a layer of sand and then a mixture, 2 inches in depth, of sand and humus collected from a bog where *Drosera* and *Sarracenia* grew. The seeds were scattered over the surface of the soil, which was sprinkled with water in order to bury them slightly. The flower pot was covered with a plate of glass, taken into a greenhouse, and placed in a concrete tank containing just enough running water to keep the soil within continuously moist. The temperature of the greenhouse was maintained at $25°-35°$ C. Under these conditions some of the seeds germinated in 10 days, some in 2 weeks, others not until the lapse of 3 weeks, and a number of them had not germinated when the experiment ended.

Mature seed

A longitudinal section of a mature latent seed (fig. 7) shows the broad face of one of the cotyledons, the stem growing point, and the hypocotyl of the embryo. The embryo is surrounded at its micropylar end by a single-layered cellular structure, which fits over it like a cap and extends to the copious endosperm. These structures are invested by a thin inner and a rather thick, black, outer seed coat.

In the writer's previous paper (25), in which the origin and development of the endosperm were traced, it was shown that the endosperm contains a bountiful supply of starch. According to later

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[1] Botanical contribution from The Johns Hopkins University, no. 110.
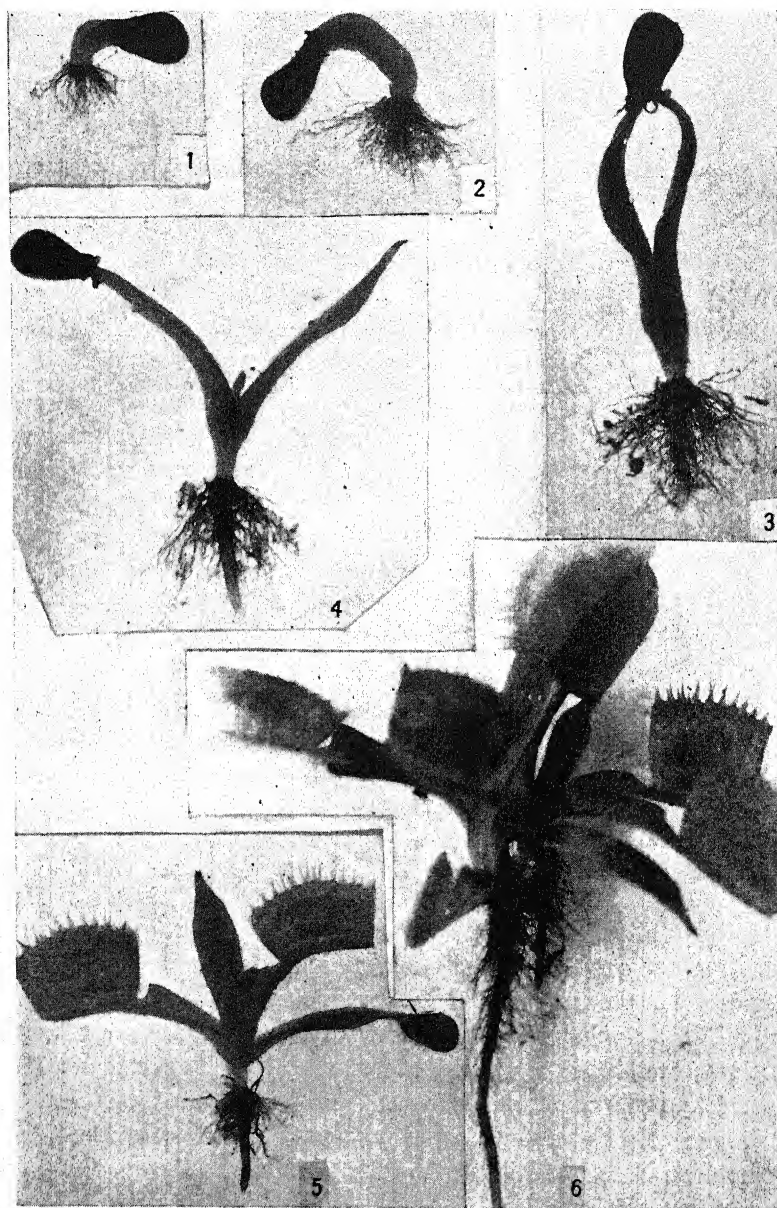
FIGS. 1-6.—*Dionaea muscipula:* stages of development in germination; ×10

study, the starch grains are usually pentagonal or hexagonal in cross-section (fig. 8), and vary from 5 to 7 μ in diameter. The persistent nucellar cells cap the micropylar end of the embryo sac, and are not encroached upon and disintegrated by the growth and expansion of the embryo and endosperm, as is the remainder of the nucellar tissue. A longitudinal section of an ovule containing an immature embryo shows the exact position of these cells, which lie above the embryo and fit closely into the micropyle (25, fig. 63). A similar section of a mature seed (fig. 7) shows these cells surrounding the embryo to the tip of the cotyledons, where they seem to connect with the endosperm. Although the nucellar cells and endosperm appear to merge into a single structure, they differ in that the former does not contain starch grains whereas the latter does, and in that they arise independently. Figs. 10 and 11, longitudinal and transverse sections through the narrow end of a germinated seed, show the large-celled nucellar tissue surrounding the two closely appressed cotyledons to the place where their tip ends are in contact with the endosperm.

The situation in *Dionaea* admits comparison with that of *Peperomia hispidula*, described by JOHNSON (13). In *Dionaea* the food is stored in the endosperm, whereas in *Peperomia* it is stored in the perisperm; in *Dionaea* the embryo is surrounded, with the exception of the tips of the cotyledons, by the persistent nucellar tissue, while in *Peperomia* it is completely invested by the endosperm. JOHNSON suggests that the endosperm of *Peperomia* serves as a medium of transfer of food between the perisperm and embryo, and that it serves as a stopper protecting the germinating seedling from loss of dissolved food, from desiccation, and from the entrance of fungi. It does not seem probable that the persistent nucellar cells in *Dionaea* function as mediums of transfer, since they do not surround the tip ends of the cotyledons. They appear, however, to form an adequate seal against the escape of dissolved food from the endosperm, and most likely prevent the entrance of fungi, as does the endosperm in *Peperomia*.

Seed germination

The first sign of germination of the seed visible from the exterior is the pushing off of the lid by the hypocotyl, at the lower end of which the primary root is developing. This lid, derived solely from
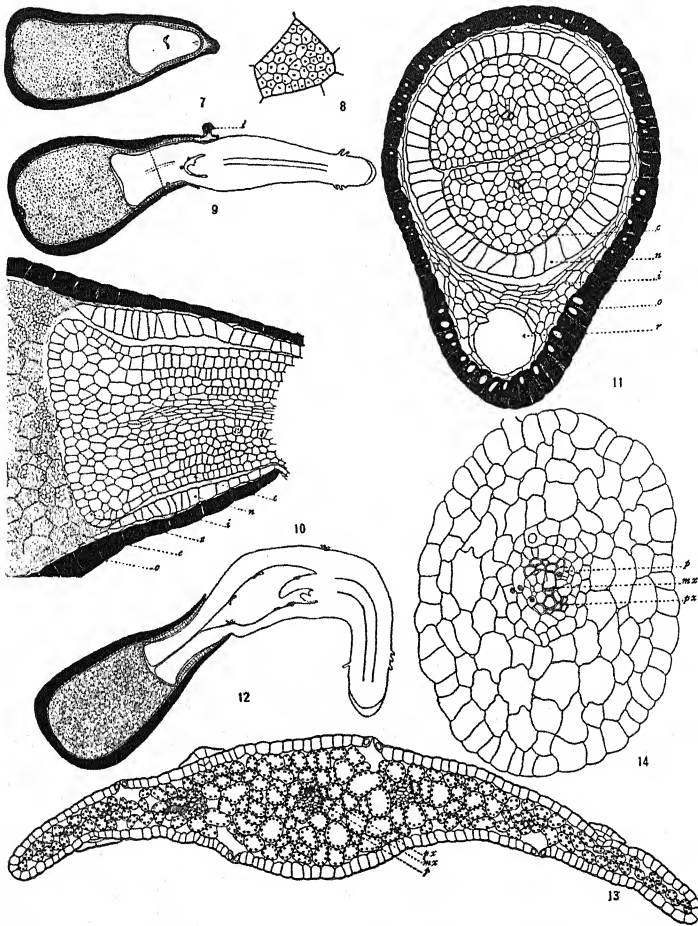
FIGS. 7–14.—Sections of seed and stages in its germination: fig. 7, longitudinal section of mature, latent seed; fig. 8, detail of single endosperm cell containing starch grains; fig. 9, longitudinal section of entire seed and seedling in early stage of germination; fig. 10, detail from fig. 9 showing end of cotyledon and tissues surrounding it; fig. 11, cross-section of seed and seedling at level indicated by dotted line in fig. 9; fig. 12, longitudinal section of seed and seedling at approximately stage shown in fig. 2; fig. 13, cross-section of cotyledon; fig. 14, cross-section of primary root (c, cotyledon; e, endosperm; i, inner seed coat; l, seed lid; mx, metaxylem; n, nucellar tissue; o, outer seed coat; p, phloem; px, protoxylem; v, cavity of vascular bundle; z, papillate epidermal cell).

the inner integument, often remains attached along one side of the seed (figs. 2, 4, 9). The short hypocotyl and the primary root grow directly downward, and the latter becomes attached to the soil by numerous dark brown root hairs (fig. 1). A longitudinal section of the seed made at this time shows that, in making their exit, the hypocotyl and primary root not only push off the seed lid, but rupture the persistent nucellar layer (fig. 9). The tips of the cotyledons possess slightly elongated, papillate epidermal cells which serve as haustoria, transporting food from the endosperm, with which they are in contact, to the median vascular bundle of their narrow, elongated, basal portion (figs. 9–11). Before the cotyledons emerge, the initial cells of the stellate hairs appear on them, and at approximately the same time they appear on the first secondary leaf developing at the stem growing point (fig. 9).

As the seedling continues its development, the basal ends of the cotyledons, as a result of intercalary growth, elongate and emerge from the seed (fig. 2), become green, and straighten up (fig. 3). The spatulate tips, which originally composed the greater part of the cotyledons, do not increase in size; they remain inclosed and continue to absorb food. A longitudinal section of the seed and seedling (fig. 12) shows the endosperm in direct contact with the tips of the cotyledons, the elongated basal portions of which are studded here and there with stellate hairs, the embryonic leaves arising at the stem growing point, the arched hypocotyl, and finally the primary root protected by the root cap.

One of the cotyledons extricates itself completely from the seed, and the two then diverge to an angle of about 90°, young leaves becoming visible between them (fig. 4). The seed remains attached to one cotyledon even after several secondary leaves have formed (fig. 5); that is, until food absorption is completed. When the seed finally drops off, the spatulate tips of both cotyledons have shriveled and turned brown. By this time the basal part of each cotyledon has reached its full size, varying from 4 to 6 mm. in length, and from 0.1 to 1.2 mm. in width. Stellate hairs and stomata appear on both their upper and lower surfaces, and a transverse section (fig. 13) shows that the epidermis is covered with a thin layer of cutin, and that

each cotyledon contains one principal vein and two smaller veins on each side, surrounded by chlorophyll-bearing parenchyma cells. The arrangement of the vascular tissues of the veins is similar to that ordinarily found in the vein of a secondary leaf of this species.

If the vascular bundles which supply the cotyledons are followed downward, it is found that as they leave the hypocotyl they are inverted, there being no transition region in the hypocotyl. Fig. 9 indicates the position, and figs. 11 and 13 show the endarch collateral arrangement of the vascular elements of the cotyledons. There is no transition region in the hypocotyl, since throughout its entire length, and that of the primary root, the vascular bundles do not alter their course, and orientation of the tissues of the bundles is similar. Since the only notable difference between the hypocotyl and the primary root is the addition of a few xylem and phloem parenchyma cells in the stele of the hypocotyl, a description of the tissues contained in one of these structures will suffice. In a transverse section of the primary root (fig .14) the following parts are to be distinguished: the epidermis, composed of one layer of cells from which root hairs may arise; the cortex, consisting of three or more layers of parenchyma cells; the endodermis; the pericycle; and the diarch xylem rays alternating with the phloem. No secondary or branch roots are formed. The growing end of the primary root is protected by a root cap, which arises from a common initial zone of the root apex, and is similar to that of the adventive root shown in fig. 33.

The greatest number of root hairs develop on the primary root slightly below the soil line, and are remarkably straight and long (figs. 1–5). They range from 0.4 to 1.9 mm. in length, and are able to resist rough handling; for when soil particles are removed from around them, even with the aid of a camel's hair brush, they escape uninjured. Inasmuch as the chemical composition of their walls seems to be identical with that of the root hairs of the mature plant, the composition of these will be discussed together later.

As the seedling continues to develop leaves, the stem apex moves from between the cotyledons to a position on a level with and external to the proximal end of the cotyledons, and by continued elongation in the same direction, a horizontal rhizome, the apex of which is protected by young leaves, is gradually formed (fig. 6). Adventitious

roots originating from the lower surface of the rhizome, soon begin to appear. Usually six to eight leaves and two or three adventive roots are formed when the seedling, consisting of cotyledons, hypo-
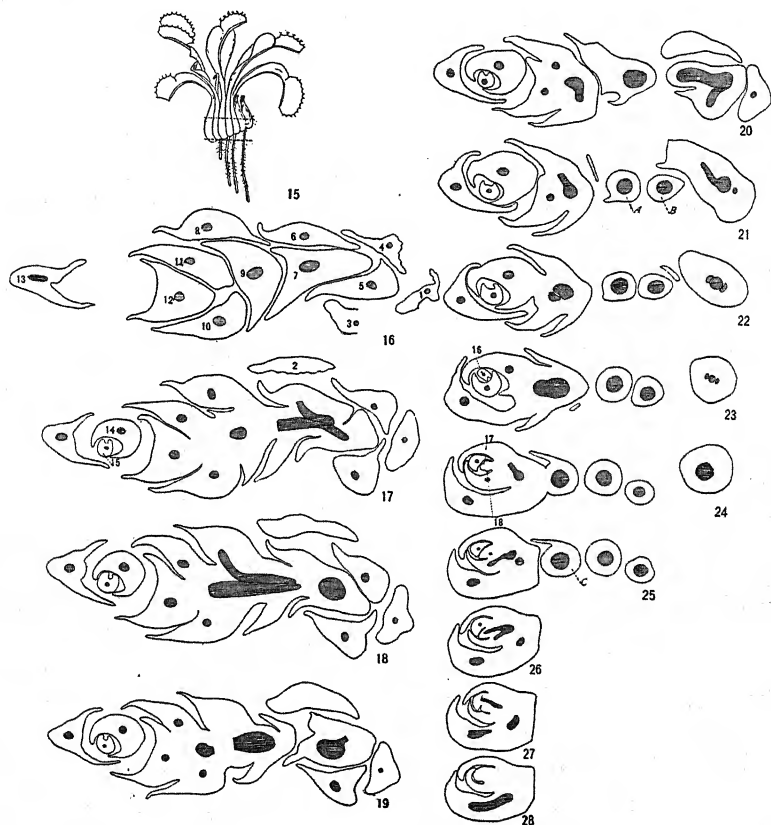
Figs. 15–28.—Fig. 15, entire seedling, showing, between dotted lines, region from which sections for figs. 16–28 were made; figs. 16–28, series of selected horizontal sections of rhizome of seedling shown, in outline, with vascular content shaded; starting with section shown in fig. 16, sections from which the 12 succeeding figures were drawn are 190, 280, 360, 460, 600, 690, 880, 1080, 1120, 1150, and 1220 μ respectively from it; leaves numbered 1 to 18 and roots lettered A to C in order of appearance.

cotyl, and primary root, begins to shrivel, turn brown, die, and is sloughed off at the proximal end of the rhizome of the now independent plant.

Mature plant

HOLM (11) has called attention to the fact that the first leaves formed, although greatly reduced in size, are identical in structure with the mature leaf, which for decades has held the interest of scientists. Since the anatomy of the blade of the leaf has been investigated and described by OUDEMANS (18), DARWIN (2), KURTZ (15), DE CANDOLLE (1), FRAUSTADT (5), GOEBEL (6), HABERLANDT (9), GUTTENBERG (8), and others, there is no necessity to discuss it here. The basal part of the leaves, rhizome, and roots will however be described. To study the relationship of these parts, 10 μ serial longitudinal sections were cut horizontally (parallel to the soil surface) through a 9-months old plant, beginning at the bases of the leaves just above the rhizome. The space between the horizontal lines in fig. 15 indicates the region of the plant from which the sections were made, and figs. 16–28 show in outline the contents of certain selected sections.

The rhizome usually grows in a directly horizontal plane, but at times the apical end is buried more deeply in the ground than the proximal end. This is particularly true of young plants. The leaves arise in close spiral succession from the apex of the rhizome (figs. 16–28). As they mature they extend first laterally and then upward, the blade and expanded part of the petiole of each unfolding in a circinate manner above ground, leaving only the base of the petiole underground. The petioles of the older leaves, whose bases overlap, inclose and protect the tender embryonic leaves. It is this close set, spiral arrangement which gives the mature plant its characteristic rosette-like appearance. Fig. 29 shows that the base, as has previously been pointed out by various investigators, also serves as a storage organ and contains an abundant supply of starch. The starch grains (fig. 30) are somewhat larger than those found in the endosperm, they are ovoid in outline, and vary from 5 to 15 μ in length. Each petiole may contain one vascular bundle, or in older plants one principal bundle and two smaller, lateral ones on each side. The endarch arrangement of the elements of the bundle (fig. 29) is that typical of the petiole of a leaf. The bundle is completely surrounded and even penetrated by parenchyma cells, which appear to contain a pectic substance.

Following the vascular bundle of any leaf to its source (figs. 16–28), it may be observed that it originates from the upper surface of the centrally located vascular tissue of the rhizome. The bases of the petioles of two adjacent leaves not only overlap but are attached, forming a storage and protective covering around the vascular tissue, which is inclosed by a layer of cells, apparently containing a pectic material, similar to that found in the leaf. The rhizome appears to consist of a composite of leaf bases with several vascular bundles coursing through them.

The flower stalk contains a dissected ectophloic siphonostele (figs. 31, 32). This indicates that a similar arrangement of the vascular tissue is entirely possible and even expected in the rhizome.

The roots originate in an almost median line from the ventral side of the vascular tissue, near the apical end of the rhizome. As each develops, it makes its way either through the region where two adjoining leaf bases overlap and are attached to each other, or, less frequently, through the enlarged middle portion of a single leaf base, carrying along a shield-shaped piece of the leaf base (fig. 34), which may be seen surrounding the proximal end of the mature root. Like the rest of the leaf base, this shield-shaped piece has stellate hairs scattered over its surface. Usually three to six functional roots, varying 2–8 cm. in length and 0.5–0.9 mm. in diameter, project downward from the rhizome.

The stele of the root of the young seedling may be diarch, as is the stele of the primary root, or tetrarch (fig. 35), whereas that of the roots of the older plants is most often octarch (fig. 36). This increase in the number of strands is possibly due, as fig. 34 seems to show, to a division of the two primary strands. A root possessing octarch bundles was described and figured by FRAUSTADT (5). Figs. 34–36 show that the roots of *Dionaea* contain a normal series of tissues: epidermis, cortex, endodermis, and stele which consists of pericycle and xylem and phloem strands. The tracheids in each vascular strand, contrary to FRAUSTADT's description, are few in number and irregularly distributed, being separated from those of the adjacent strand by xylem parenchyma. A dark staining material, similar to that in the cells surrounding the vascular tissue of the rhizome and leaf, is found in some of the cells of the cortex, endodermis, and

pericycle, and frequently in some of those investing the phloem. In older roots the epidermis and a part of the cortex may be sloughed off, and, when this happens, this dark-staining material appears to collect in the cells of the pericycle on the side where the cortex is
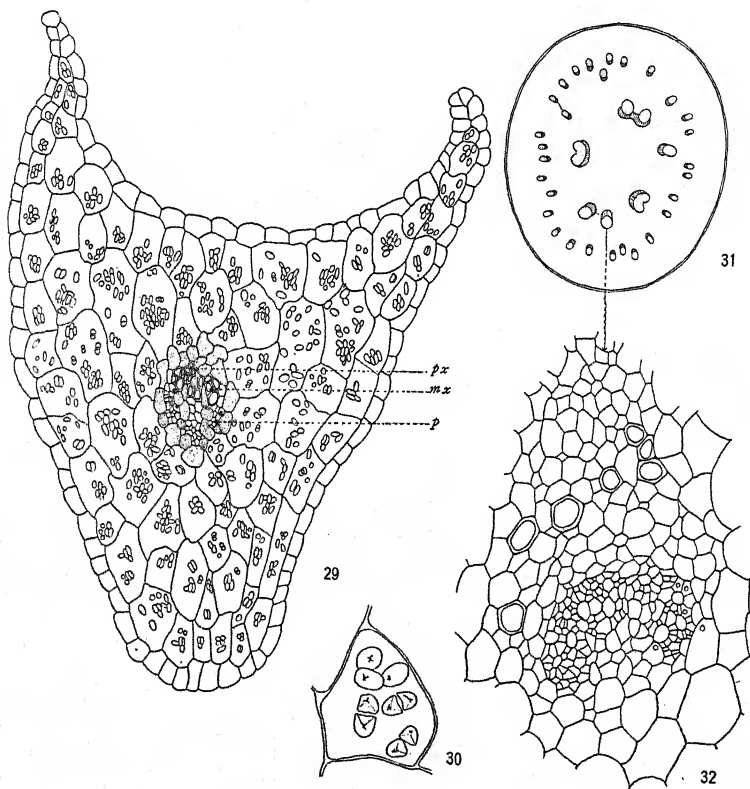
FIGS. 29–32.—Fig. 29, detail of leaf petiole no. 12 in fig. 16; fig. 30, detail from fig. 29 of single starch-containing parenchyma cell; fig. 31, cross-section of flower scape showing arrangement of vascular bundles; fig. 32, detail of single bundle from flower scape (mx, metaxylem; p, phloem; px, protoxylem).

sloughed off (fig. 36). The growing end of the root is protected by a root cap (fig. 33), which originates like the root cap of the primary root from a common initial zone of the promeristem. A few millimeters behind the growing point, root hairs begin to form which, like those of the primary root already described, soon become thick-

walled and turn brown. They may persist throughout the life of the root, and may even be found on the old roots which have ceased to function and are being sloughed off the proximal end of the rhizome.
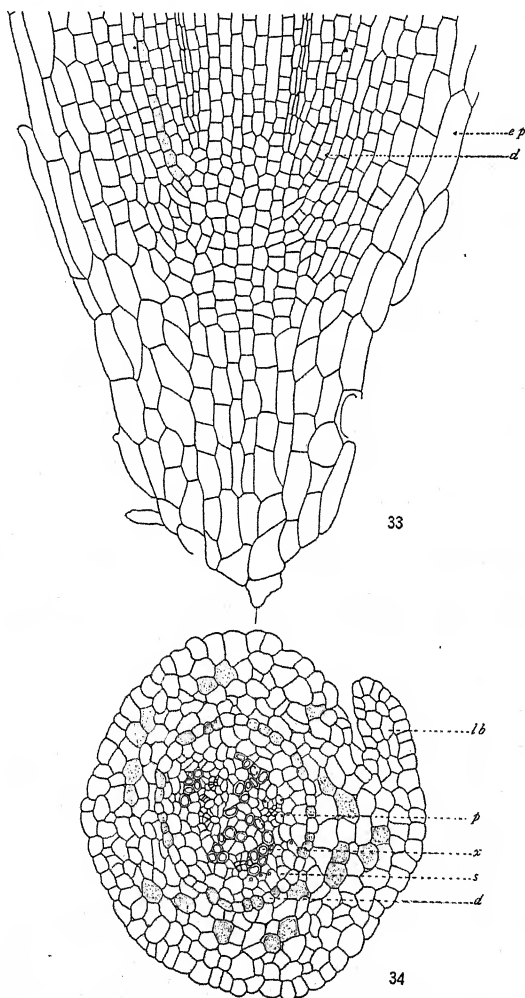
FIGS. 33, 34.—Fig. 33, longitudinal section of root tip showing root cap arising from common initial zone; fig. 34, detail of root *A* in fig. 21, showing 4 groups of tracheids separated by xylem parenchyma, two distinct phloem strands, and third phloem strand in process of separation (*d*, endodermis; *ep*, epidermis; *lb*, portion of leaf base; *p*, phloem; *s*, pericycle; *x*, xylem).

In an effort to ascertain their composition, the cell walls of the
root hairs were tested microchemically as follows: for cellulose, with
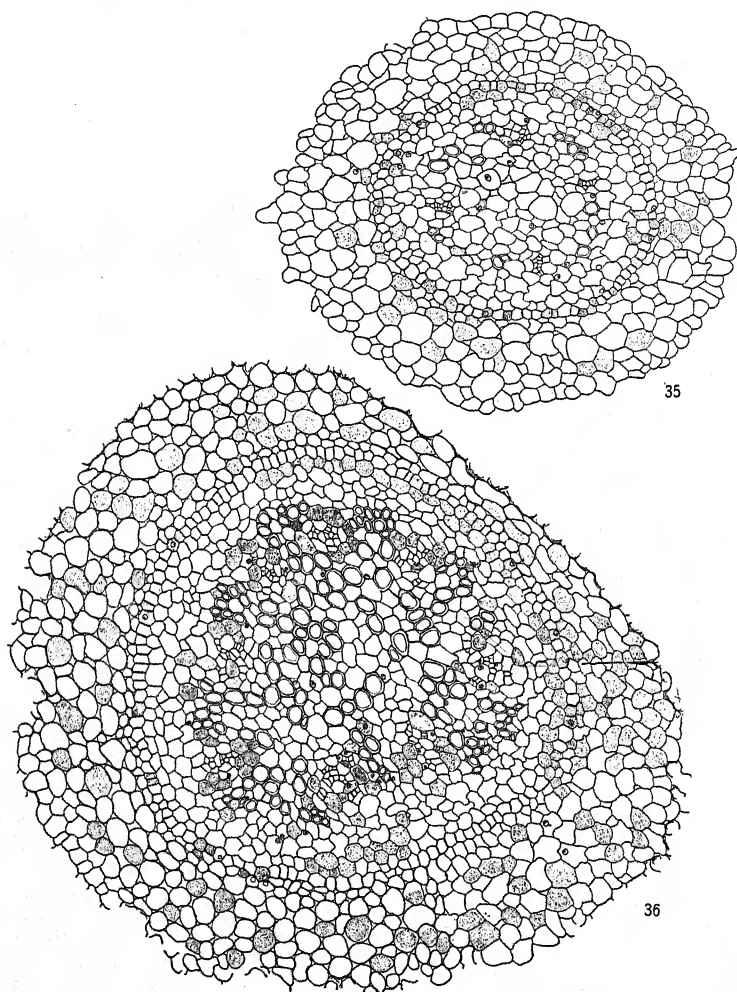
FIGS. 35, 36.—Fig. 35, cross-section of root slightly older than one in preceding
figure, showing fewer tracheids, more parenchyma, and 4 separate phloem strands;
fig. 36, cross-section of octarch root from old plant, showing epidermis and part of
cortex sloughed off.

concentrated sulphuric acid, fresh cuprammonia, iodine and sulphu-
ric acid, and with chloriodide of zinc both before and after treatment

with nitric acid and potassium chlorate; for lignin, with phloroglucin and hydrochloric acid, and with aniline sulphate and sulphuric acid both before and after bleaching with sodium hypochlorite; for callose, with resorcin blue; for pectose, with ruthenium red, hydrochloric acid, and potassium hydroxide; for calcium pectate, with ammonium oxalate; for suberin and cutin, with alkanin, chlorophyll, cyanin, and Sudan III, before and after treatment with both potassium hypochlorite and potassium hydroxide. Although the root hairs of *Dionaea* responded to none of these tests, which include those normally used for determining the composition of the cell walls of root hairs, they were finally dissolved after 2 hours' gentle boiling in either a 50 per cent solution of chromic acid or in Schulze's maceration fluid, or after remaining in potassium hypochlorite for 3 full days. Since chromic acid is a recognized test for cutin, and since the root hairs of *Dionaea* responded to this test, they probably contain this substance, although the other tests failed to reveal its presence. The root hairs of *Dionaea*, therefore, appear to be vestigial structures which have ceased to function.

Systematic position of Dionaea muscipula

There is some diversity of opinion among recent taxonomists as to the position of the Droseraceae. ENGLER and GILG (4) and RENDLE (21) include the three families Sarraceniaceae, Nepenthaceae, and Droseraceae in the order Sarraceniales. HUTCHINSON (12) includes the Sarraceniaceae and Droseraceae in the Sarraceniales, but places the Nepenthaceae, because of their woody structure, in the order Aristolochiales. DIELS (3), monographer of the Droseraceae for *Das Pflanzenreich*, in discussing the relationship of this family, attaches particular importance to the hypogynous insertion of the parts of the flower, and the truly parietal placentation of the ovules in *Drosera*, and therefore places the Droseraceae under the order Parietales, nearest akin to the Violaceae.[2] The same scheme is followed by WETTSTEIN (26), who, however, places the Sarraceniaceae and Nepenthaceae in a separate order, the Polycarpicae. While these taxonomists agree in regarding the four genera, *Drosophyllum, Dio-*

[2] DIELS gives an account of the different positions in which the Droseraceae had been placed by taxonomists up to 1906.

naea, Aldrovanda, and *Drosera,* as composing the family Droseraceae, SMALL (**24**) places *Dionaea* in a separate family, the Dionaeaceae. It is of interest to see what light the features of the development of *Dionaea muscipula* recorded in the present paper, as well as in the earlier paper (**25**), throw on its position in the plant kingdom.

In *Dionaea,* as in *Drosophyllum,* 10–20 (most often 15) stamens are formed, whereas in both *Drosera* and *Aldrovanda* only five are developed. The nuclear phenomena of the microsporocytes of *Dionaea* are essentially like those of *Drosera rotundifolia* and *D. longifolia* described by ROSENBERG (**22**). Furthermore, judging from RO-SENBERG's figures, the chromosomes of *Drosera* appear to be similar in shape and form to those of *Dionaea.* He gives 10 and 20 as the haploid number of chromosomes respectively in *D. rotundifolia* and *D. longifolia,* whereas 15 is the haploid number in *Dionaea.* The pollen grains of *Dionaea* hang together in tetrads, a characteristic of the entire family. In structure they appear to be similar to the pollen grains of *Drosera squamosa* as described by DIELS (**3**).

One of the most distinctive parts of the flower of the Droseraceae is the pistil. In *Dionaea* and *Drosophyllum* it is composed of 5 carpels, and contains numerous seeds on a basal placenta. Whether the ovary of *Drosophyllum* is "paracarpous," and bears its ovules without the aid of the vegetative apex of the flower, as described by GOE-BEL (**7**) for *Dionaea,* or whether the vegetative apex shares in the formation of the ovary, has not been reported, nor has the writer had an opportunity to investigate this situation. Judging from DIELS' figures, however, one would be inclined to conclude that they develop as in *Dionaea.* In *Aldrovanda* and in *Drosera,* the ovary is syncarpous, in the former consisting of 5 carpels, in the latter 2–5 (most often 3). In both genera the ovules develop on marginal parietal placentas.

While all four genera have anatropous ovules, there are striking similarities as well as differences in the development of the ovules of *Dionaea* and of *Drosera.* In *Dionaea* the hypodermal archesporium divides to form two or sometimes three layers of parietal cells above the embryo sac, whereas in *Drosera* (PACE **19**) parietal cells are commonly not formed, although sometimes a single layer develops between the epidermis and the mother cell. Furthermore, the enlargement of the outer layer of nucellar cells of *Dionaea* resembles that

of *Drosera rotundifolia* described by Miss PACE, but the fate of the
cells in *Dionaea* (25) is different from that suggested by her for
Drosera. Moreover, a comparison of the mature embryo sac of *Dio-
naea* (25) with that of *Drosera* (19) shows the two to be similar.
Dionaea develops a seed lid not markedly different from that in
D. rotundifolia described by NETOLITZKY (17).

Dionaea is the only one of the Droseraceae in which the persistent
nucellar tissue has been observed and described. The slides of *Dro-
sera rotundifolia* made by PETERS (25), when compared with slides
of *Dionaea*, show that the endosperm of the two passes through
practically the same stages of karyokinetic differentiation and de-
velopment. The embryo of *Dionaea*, as is true of that of the other
members of the family, is small, and lies at the base of the seed in
direct contact with the copious endosperm.

A comparative study of germination of the seeds of the four
genera of the Droseraceae shows the following gradation in the de-
velopment of the primary root and cotyledons. (1) Neither *Aldro-
vanda*, as described by KORZCHINSKY (14), nor *Drosera*, as described
by HEINRICHER (10) and later by DIELS (3), develops a vigorous
primary root; instead, each forms a rudimentary root which func-
tions for a brief time only. (2) *Dionaea*, according to HOLM (11),
develops a primary root, which, as the present investigation shows,
ceases to function and is sloughed off with the cotyledons after
leaves and adventitious roots have formed and the plant has become
independent. (3) *Drosophyllum*, according to PENZIG (20), develops
a primary root which persists. On the other hand, the four genera
vary with respect to the function and development of the cotyledons:
in *Aldrovanda*, according to KORZCHINSKY (14), and in *Drosophyl-
lum*, according to PENZIG (20), the cotyledons remain within the
seed and absorb food from the endosperm; whereas in *Drosera*, ac-
cording to HEINRICHER (10) and DIELS (3), and in *Dionaea*, only the
tips of the cotyledons do this. The remaining and greater part of
the cotyledons emerge and carry on the process of photosynthesis.
Germination of the seed of *Dionaea* closely resembles that of *Sar-
racenia* and *Darlingtonia* described by MACFARLANE (16).

Since there are numerous characters upon which the four genera
agree exactly, since there is a smaller number upon which at least
two genera agree, and since there are only a few upon which all the

genera differ, it seems logical to concur with those who place *Dionaea* among the Droseraceae, and divide the family into the four genera *Drosophyllum*, *Aldrovanda*, *Dionaea*, and *Drosera*. Furthermore, a comparative study of the morphology of the flowers of the Droseraceae and the Sarraceniaceae and Nepenthaceae, although showing that these differ as to number of stamens, number of carpels, and placentation of ovules, definitely indicates their affinity. Among the Sarraceniaceae the stamens vary from 10 to ∝; in *Sarracenia* there are 70–80, which, according to SHREVE (**23**), originate as ten groups of primordia; in *Darlingtonia*, according to MACFARLANE (**16**), 15 is the typical number formed. Among the Droseraceae, on the other hand, the stamens range from 5 to 20; in *Dionaea* and in *Drosophyllum* there are 10–20 (most often 15), which, in *Dionaea* and probably in *Drosophyllum*, originate as in *Sarracenia* as ten groups of primordia; in *Drosera* and in *Aldrovanda* 5 are regularly produced. The pistil shows the following variations: in *Aldrovanda* it consists of 5 and in *Drosera* 2–5 (most often 3) united carpels, which bear their ovules on marginal, parietal placentas; in *Drosophyllum* and in *Dionaea* it is made up of 5 carpels, which develop ovules on their united upraised bases; in *Sarracenia* and *Darlingtonia* it has 5, and in *Heliamphora* 3 united carpels, which grow inward and develop their ovules on axillary placentas. Apparently, therefore, the three families should be placed in the order Sarraceniales.

Summary

1. Germination of the seed and development of the seedling of *Dionaea* proceed as follows: the hypocotyl and the primary root elongate, rupture the nucellar cap, and push aside the seed lid; the cotyledons partially emerge, turn green, and straighten up, carrying the seed with them; one cotyledon releases itself completely and the other remains attached by its tip end, the two then diverging and young leaves appearing between them. The stem apex, which gives rise to leaves in spiral succession, gradually moves horizontally from between the cotyledons, forming a subterranean rhizome from the lower surface of which adventive roots arise; the cotyledons, hypocotyl, and primary root finally cease to function and are sloughed off the basal end of the rhizome of the now independent plant.

2. The main aspects of the anatomy of the seedling are as follows: the hypocotyl and the primary root are similar in structure, both having epidermis, cortex, endodermis, pericycle, and a diarch stele; there is no transition region in the hypocotyl, the vascular bundles being inverted where they enter the cotyledons; the cotyledons, with stomata and stellate hairs present on both upper and lower surfaces, have a median collateral endarch vascular bundle from which two or more lateral branches extend.

3. The rhizome appears to consist of an agglomeration of overlapping undiverged leaf bases with several vascular bundles coursing through them.

4. The stele of the root of a young plant is usually tetrarch,whereas the stele of the root of a mature plant is most often octarch.

5. The root hairs, which begin to form a few millimeters behind the growing point, soon turn brown and become thick-walled, often persisting throughout the life of the root. Of the microchemical tests used to determine the composition of their cell walls, the chromic acid test for cutin alone gave positive results. They appear, therefore, to be vestigial structures.

6. The evidence gained from a comparative study of the development of *Dionaea* and the plants closely related to it indicates that *Dionaea* should be placed in the family Droseraceae and in the order Sarraceniales.

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ASCENT OF SAP IN PLANTS

FANNY SMITH, R. B. DUSTMAN, AND CHAS. A. SHULL

(WITH ONE FIGURE)

The ascent of sap in plants, particularly tall plants, was long a puzzle to botanists and physicists. Finally a satisfactory explanation of the lifting of water in even the tallest trees was given by the work of DIXON (4), ASKENASY (1), RENNER (7), BODE (2), and others (6, 9) during the last 35 years. The explanation is given in terms of such forces as the evaporational power of water, the surface tensional and imbibitional forces of the cell colloids of the leaf, the osmotic behavior of water in the presence of semipermeable or differentially permeable membranes (especially protoplasmic membranes), and the cohesional properties of the tracheal sap. All of these forces have been found adequate for the work. The average force of water evaporation into a complete water vacuum has been estimated at 1350 atmospheres; the imbibitional forces developed by drying organic matter range from zero to about 1000 atmospheres, depending on the degree of drying; osmotic forces up to 205 atmospheres (10) have been found in the living cells of leaves; and the cohesion of water is probably not less than 300 to 350 atmospheres. Since the total force required for lifting water to a height of several hundred feet is estimated at approximately 20 atmospheres, these forces can all operate at modest fractions of the possible development of power. In view of these facts, the cohesion theory of sap ascent has been rather generally accepted as the most reasonable explanation of sap rise phenomena.

One recent author, however, holds to another theory. BOSE (3) believes that the water is pumped upward through the inner cortical regions of the stem by pulsating cells in which hydraulic waves of contraction and relaxation succeed one another up the stems of the plant. This idea of BOSE's is probably based upon a complete misconception of the nature of certain electrical phenomena which he

claims to have observed, and which he has interpreted in terms of pulsations similar to that of a beating heart.

It is interesting to note that both Bose and Dixon propose a vitalistic cause of sap ascent. Bose's views can be laid aside without discussion, since his location of sap ascent in the cortex is contrary to the experience of all others who have worked upon this problem. Even if others were able to demonstrate the electrical disturbances Bose claims to have observed, there is no reason for conceding the interpretation which he has placed upon them.

A different theory of vital action is proposed by Dixon. He assumes that the chief source of energy available and responsible for the rise of the tracheary columns of water in woody stems is the energy of respiration. He ascribes the removal of water from the leaf cells to an active secretion, which takes place irrespective of any evaporation which occurs into the leaf interspaces. Dixon admits that evaporation may exceed secretion, and in such exceptional cases might exert an evaporational pull upon the water columns; but the ordinary and usual water ascent is brought about by the active secretion, after which the water is evaporated, without the evaporation having any part in the development of the forces of water rise. This idea of an active secretion of water by the leaf is concurred in by Bose, who describes the active secretion of water by the leaves of *Nauclea*. Bose covered the leaves of *Nauclea* with vaseline, and claims that in spite of this covering the leaves kept on taking in water at the cut end of the petioles, and excreting it as liquid water under the coating of vaseline, since there was no opportunity for evaporation under these conditions.

As evidence for his rather unusual theory of active secretion of water by leaves, Dixon describes and figures experiments with branches cut under water and mounted in an inverted bell jar, in such a manner that the upper part of the branch was completely submerged in water. These operations were all carried out in such a way that the cut surfaces of the stem were never exposed to the atmosphere, in order that the water columns of the stem might not be broken, and that there might not be any clogging of tracheae with air bubbles.

The lower end of the cut stem projected through the cork which

closed the bell jar below, and was allowed to dip into a solution of eosin. Since the leaves were all submerged, there could be no evaporation of water from the leaf surfaces. In the work to be reported later the same precautions were taken to prevent entry of air into the cut stems.

Although the transpiration was stopped in Dixon's experiments, he found a continuous rise of eosin through the stem; and he used this intake of eosin at the cut end of the stem as crucial evidence of the continuous excretion of liquid from the leaves into the surrounding liquid which bathed the shoot. This rise of eosin in the branch occurs against the hydrostatic pressure upon the plant from the depth of the water in the bell jar, and Dixon ascribes to respiration the generation of the energy used to cause this excretion against the head of pressure in the bell jar.

Some years ago one of the writers (8) commented upon these observations by Dixon, but without going into a detailed criticism. It seemed obvious that saturation deficit in the tissues of the stems used was not sufficiently taken into consideration. Normal translocation was afterwards associated with saturation deficit effects by Dixon (5) in his work with potatoes, in which he described a downward translocation of sap in the tracheae, and proposed that the xylem could transfer water upward, and organic foods downward, at the same time. The present paper indicates that the rise of eosin solution observed by Dixon in his bell jar experiments may be explained solely on the grounds of a saturation deficit existing in the leaf and stem tissues at the time the branches were cut. Such a deficit must always exist in growing plants, and unless precautions are taken to relieve it, it will manifest itself in the results of such experiments. Although submerged in water, the internal water deficit continues to determine the intake of water until the deficit is satisfied. This will occur through the paths of least resistance, which in the case of a submerged plant is through the cut ends of the tracheae in the eosin bottle. Tests with various plants showed that one could easily repeat Dixon's experiences if the stems were cut under water and set up at once in the bell jar. But Dixon seems not to have made adequate provision for overcoming this saturation deficit in the leaves and branches used in his experiments. Our pre-

liminary experiments, run in the late autumn of 1922, were designed
to take care of this important factor in the rise of eosin. Later the
work was extended at intervals to a number of other kinds of plants
during the summers of 1923 and 1926, and to include studies of
transpiration with BOSE's bubbler. The latest studies involved the
use of winter twigs during the latter part of the winter of 1930.

Materials and methods

In the first experiments (DUSTMAN) several cottonwood branches,
fuchsias, and a shoot of *Grevillea robusta* were used. All shoots were
20–30 cm. in length, the cottonwood stems being cut in mid-Decem-
ber, and having large well developed winter buds. This species was
chosen because of the large buds and consequent abundance of re-
spiring material. The fuchsias and *Grevillea* plants were potted speci-
mens from the University greenhouses, and were chosen for their
large leaf areas and relatively rigid stems. The diameter of the cot-
tonwood stems was approximately 0.75 cm., and of the others about
0.5 cm. All were cut under water, and kept continuously under water
until the conclusion of the experiment. The fuchsia and *Grevillea*
stems were mounted in inverted bell jars, and the cottonwood stems
in inverted 2-liter bottles which were completely filled with water.

In order to overcome, so far as possible, any saturation deficit
in the leaves and stems, the potted plants were placed with their
roots in pans of free water beneath closed bell jars for 24 hours before
the branches were cut. The condensation of free water on the sides
of the bell jars indicated that this had largely been accomplished.
The stems were then cut under water, mounted in the inverted
bell jars, and, to insure saturation, allowed to stand completely sub-
merged for a time before the eosin was added. The cottonwood twigs
were not placed under bell jars previously, but were permitted to
stand submerged in the bottles for 24 hours before being tested with
eosin. Later results showed that this is not a long enough period to
secure complete satisfaction of the saturation deficit, in the case of
dormant twigs.

That the cottonwood branches contained a considerable volume
of gas, and that this gas was under some pressure, was shown by the
fact that when cut the stems emitted a continuous stream of tiny
bubbles for a short time. This release of gases was again apparent

when the stems were fitted into the bottles. Two factors may explain the emission of gas: rise in temperature when first brought in and hydrostatic pressure when finally set up for the experiment. The hydrostatic pressure was in part due to the set up, for the bottles were completely filled with water, and the rubber stoppers through which the stems passed were fitted tightly into the bottle necks. In the most recent experiments with winter twigs, they were used in inverted bell jars so that the eosin intake would not have to take place against the compressibility of water and the elastic give of the rubber cork. The results of the later work are in harmony with those of the preliminary cottonwood twig experiments.

The later work (SMITH), during the summers of 1923 and 1926, involved several types of experiment: (1) transpiration of branches with and without saturation deficit into saturated atmosphere; (2) secretion experiments with submerged plants some of which had been relieved of water deficit and others not; and (3) secretion experiments with leaves, using BOSE's bubbler method with vaseline-coated leaves.

The studies of sap rise in a saturated atmosphere were made with a variety of species. Young and vigorous branches of the plants listed in table I were used. They were cut under water, and the cut ends kept there for 20 minutes in the dark room. Then they were transferred to a bell jar in which the atmosphere was kept as nearly saturated as possible, and still kept standing in water for an hour to give opportunity for overcoming the water deficit of leaf and stem tissues. At the end of an hour the water around the ends of the cut stems was replaced with eosin solution, and a beaker of water at 100° C. was placed within the bell jar, which was now closed and returned to the dark room. The rise of eosin was measured at the end of one hour. Similar experiments were performed with branches which had not been relieved of their saturation deficit.

For the secretion experiments the plants listed in table II were used. These were cut under water and arranged in the bell jar just as in the preliminary tests. The cut ends of the stems were kept covered with water for 2 hours, the shoot being submerged, at which time the water was replaced by eosin. After an hour the ascent of eosin in the stems was measured.

The experiments with BOSE's bubbler were performed with

healthy leaves of the plants listed in table III. The leaves were severed from the plant under water, and left for 30 minutes with the petioles in water. The leaf petiole was then passed through one of the holes of a 2-hole stopper, and sealed in air-tight with wax. During these operations the cut end of the petiole was never allowed to get out of the water. The bubbler tube was inserted tightly through the second hole of the stopper, after which the stopper was inserted into the water reservoir with the petiole projecting into the water, and sealed with an air-tight seal.

Typewriter oil or clove oil was used as the indicator valve for the bubbler. The rate of transpiration is determined by the number of bubbles of air which pass the oil valve in a definite time interval. After the set-up had stood for one hour, to give the leaf time to dry and become adjusted to the conditions of the experiment, and to determine the normal rate of transpiration while uncoated, the leaf was carefully and thoroughly coated with vaseline. The readings were extended over a period of 30–60 or more minutes after the leaves had been coated.

The latest studies with winter twigs (SHULL) were made with stems cut under water, and kept there for various periods of time after being arranged in the bell jars. Care was taken to avoid great changes of temperature by having at hand considerable volumes of water which had come to room temperature. The factor most likely to give trouble in such experiments is the internal atmosphere of the tracheal system. Slow solution of the gases in water on standing is likely to occur, with some rise of eosin. Any lowering of temperature will cause contraction of gases, and the registration of some rise of eosin; for even if later on the gas is expanded to its original volume, the eosin has stained the xylem as far up as the contraction of gases drew it.

Twigs of *Ulmus americana, Ptelea trifoliata, Rhus canadensis, Cornus stolonifera* (?), and *Crataegus* sp. were used. After a 12-hour period with the cut ends in eosin, the twigs were split, and observations made of the rise of eosin into the tracheal system.

Results

The preliminary experiments indicated that the rise of eosin was probably controlled by saturation deficits in the tissues. The cotton-

wood branches, containing air, are very difficult to saturate, and during slow solution of the internal gases some eosin would enter the stems, owing to this cause and to slight fluctuations of temperature. One twig, submerged 24 hours and allowed to stand in eosin solution for 19 hours, showed only 2 cm. rise. Two others, submerged for 48 hours and then arranged in eosin, showed in the first of the two a rise of 5.5 cm. in 2 days, and in the second a rise of 5.0 cm. in 5 days. One would expect a much greater rise in 5 days than in 2 days, if the rise were due to continuous excretion of water. The three controls,

TABLE I

RISE OF SAP OF PLANTS IN SATURATED AIR

PLANTS USED	LENGTH OF BRANCH (CM.)	TREATMENT			RISE OF EOSIN (MM.)
		Time in H₂O in dark (min.)	Time in H₂O in bell jar (hours)	Time in eosin (hours)	
Quercus macrocarpa.....	27	20	1	1	2
Ribes odoratum.........	30	15	1	1	3
Philadelphus grandiflorus.	25	15	1	1	2
Ulmus americana........	30	20	1	1	2
Ailanthus glandulosus....	47	20	1	1	3
Ginkgo biloba..........	31	20	1	1	4
Robinia pseudacacia.....	34	20	1	1	3
Catalpa speciosa........	36	20	1	1	2
Tilia americana.........	30	20	1	1	3
Acer saccharinum.......	35	20	1	1	3
Platanus occidentalis....	43	20	1	1	2

allowed to stand in air instead of being submerged, and then in eosin for the same length of time (19 hours, 2 days, 5 days), showed 13 cm., 16.5 cm., and complete traversing of the stem respectively.

The fuchsias, prepared as previously described, showed only 1 mm. rise in 19 hours, and only 1 mm. in 48 hours respectively, for the two stems tested. And the *Grevillea robusta*, kept in the eosin solution for 5 days after the saturation deficit was relieved, showed a rise of only 1 mm. This same stem was then freshly cut and exposed to air for 5 hours, standing in eosin. The rise of the eosin was 30 cm. during that period. The small rises noted in the fuchsias and *Grevillea* are easily accounted for by diffusion alone.

The experiments designed to test the rise of sap in plants kept in a saturated atmosphere are shown in table I.

The branches used varied in length from 25 to 47 cm., and the rise

of eosin in the cut stems was in no case over 4 mm. in an hour. In the majority of cases it was only 2–3 mm. One must believe that this small rise is due mainly to diffusion of eosin into the stem, and not to active secretion of water from the leaf.

In numerous other experiments the branches were set up in a saturated chamber with the ends dipping into water for 3 hours. At this time the water was replaced with eosin for 12–24 hours, and the rise noted. These branches were then removed and allowed to wilt for 1.5–2 hours, after which they were returned to the eosin, and the tops exposed in a saturated atmosphere under a bell jar. Ten species were employed. Not a single shoot showed significant rise of eosin during the first period under the bell jar; but after wilting, all of them showed rise on account of the saturation deficit induced during wilting. The largest rise occurred in *Ginkgo* and *Philadelphus*, which showed 15 cm. rise during the 24 hours; the smallest was in *Cornus florida*, which showed a rise of 1.5 cm. The average rise of duplicate experiments with all ten species was 7.36 cm. These results are in strict agreement with those of table III, and indicate the significance of the saturation deficit in connection with sap movement.

Fifteen species of plants were used in the experiments with submerged shoots. One branch was always set up in air to show the rise of fluids in the branch during normal transpiration, where evaporation accompanies sap rise. The results are presented in table II. As is seen from the data, when the plants were submerged, and given one or two hours to imbibe water before applying eosin to the cut stems, there was in no case any significant rise of sap.

The greatest rise, 5 mm., was found in *Ribes odoratum*, and in most cases the eosin had penetrated only 2 or 3 mm., which might be expected as the result of diffusional entry. We were unable to obtain any evidence confirmatory of the secretory theory of sap ascent, for whenever the saturation deficit was really satisfied before the eosin was applied, there was no significant sap movement in the stems.

The last two columns of table II present the results obtained when control stems are set up in air. The contrast with the rise in stems relieved of saturation deficit is evident. In a number of instances, after standing surrounded by water with no appreciable rise of

eosin, the water was removed from the bell jars and the plants exposed to air with the cut ends still dipping into eosin. Every shoot showed considerable rise, and in some branches the dye penetrated to the tips of the shoots.

Branches were also placed in the bell jars, immersed in water, and given the eosin at once, without giving an opportunity to satisfy the water deficit existing in the twigs. There was always a rise of the

TABLE II

ASCENT OF SAP IN SUBMERGED SHOOTS

PLANTS USED	LENGTH OF BRANCH (CM.)	TREATMENT			RISE OF EOSIN (MM.)	RISE IN CONTROL	
		Time left in H_2O (hours)	Temp. of H_2O (° C).	Time in eosin (hours)		(cm.)	(min.)
Quercus macrocarpa.....	38	2	23	1	3	35	28
Ribes odoratum.........	29	1	21	1	5	33	39
Philadelphus coronarius..	34	1	20	1	3	22	26
Chrysanthemum							
Wm. Turner..........	31	2	21	2	2	30	30
Black Hawk..........	27	2	21	2	2	28	27
Chrysolaria..........	40	2	20	1	3	9.5	30
Ulmus americana.......	49	1	20	1	2	38	29
Ailanthus glandulosus ...	43	1	21	1	2	41	30
Ginkgo biloba..........	32	1	20	1	3	29	45
Robinia pseudacacia.....	42	1	20	1	2	29	30
Catalpa speciosa........	48	1	20	1	3	30	30
Lycium vulgare.........	28	1	21	1	2	28	25
Philadelphus grandiflorus	35	2	20	2	4	32	30
Tilia americana.........	34	1	20	1	2	15	35
Ptelea trifoliata.........	49	1	20	1	3	16	28

eosin, in harmony with DIXON's results with experiments set up in the same manner.

Finally we present the results of repetitions of BOSE's work with the bubbler. Active leaves were selected for the work. *Hydrangea arborescens* leaves gave a reading of eleven bubbles in 61 minutes. After coating both sides of the leaf with boiled and cooled vaseline, one bubble was noted at the end of 28 minutes, and no more during the next hour. No water was found under the vaseline. The temperature was 24° C. during the test. The general results with leaves of ten species of plants are given in table III.

In 70 per cent of the cases no bubbles were produced after the vaseline was applied. In 30 per cent one bubble was noted after

considerable lapse of time. In not a single instance was any water found under the vaseline coating, even at the end of 24 hours. In the case of the most active leaf, *Vitis labrusca*, bubbling ceased immediately with the covering of the leaf with vaseline, and no sign of water secretion beneath the vaseline could be observed.

The results recorded in table III refute the claim of BOSE that transpiration is a secretory process. Unfortunately it was not possible to use the leaf of *Nauclea* for this experiment, as the plant is not native to this country and could not be found among exotic collec-

TABLE III

RESULTS WITH BUBBLER METHOD WITHOUT AND WITH VASELINE COATING

PLANTS USED	LENGTH OF OBSERVATION WITHOUT VASELINE (MINUTES)	No. OF BUBBLES	No. OF BUBBLES AFTER COATING WITH VASELINE	WATER UNDER VASELINE	TEMPERATURE (° C.)
Hydrangea arborescens..............	61.0	11	1 after 28 min.	None	24
Begonia sp...........	70.5	14	None	"	24
Ptelea trifoliata......	67.3	8	1 after 27 min.	"	25
Cornus florida.......	73.0	11	1 after 11 min.	"	29
Catalpa speciosa.....	51.6	5	None within 1 hour	"	31
Tilia américana......	58.8	18	" " " "	"	32
Philadelphus grandiflorus.............	57.5	10	" " " "	"	32
Vitis labrusca........	26.0	22	" " " "	"	28
Ampelopsis quinquefolia..............	64.4	6	" " " "	"	28
Viola palmata.......	53.0	7	" " " "	"	29

tions in local conservatories. Certainly none of the species examined behaved in the manner described for *Nauclea*. Even if BOSE's observations of the action of *Nauclea* leaves were found to be correct, it cannot represent a general behavior among plants.

The most recent tests, made with twigs in winter condition during the latter part of the winter of 1930, were in harmony with the earlier results with cottonwood twigs. These later experiments, however, did not confine the twigs in a tight bottle. They were set up in bell jars, submerged in water as were many of the shoots previously described. It was found that twigs set up at once with eosin, without overcoming saturation deficit, continued to absorb the dye for considerable periods of time. The longer they were submerged in water

and supplied with water at the cut ends before the eosin was applied, the less the dye was absorbed. When left in water sufficiently long, the rise of eosin was only 2–5 mm. in a 24-hour period. It was necessary to take precautions in regard to temperature changes, as a lowering of the temperature during the experiment led to contraction of internal gases and absorption of the dye. With controlled conditions, however, it was demonstrated that the rise of sap in winter twigs is a function of the saturation deficit, and does not depend on a secretory or excretory process.

Discussion

The results obtained in the simple experiments recorded here indicate that sap ascent is controlled, not by the secretion of water by the living cells of the leaf, but by the evaporation of water from the leaf. When evaporation is stopped, and the previously developed saturation deficit is relieved or satisfied, sap ascent ceases. There seems to be no good reason for ascribing to the transpiring cells a special secretory function in connection with the rise of water through the plant. It is true that living cells are necessary to the process of sap rise through prolonged periods of time, although there must be considerable water ascent in dead trees, due to purely physical causes. The main function of living cells is presumably in connection with the conversion of imbibitional forces into osmotic forces, development of osmotic action being necessary to the normal development of a pull upon the water in the tracheal tubes. If the living cells of the leaf are killed, they are then unable to transfer water from cell to cell, and the leaf dries out. The dead cell-wall colloids may for a long time continue a slow water loss, with some water rise, mainly through the stem; but the rapid transpiration stream as it exists in living plants is no longer present.

It does not seem probable that respiratory energy is needed for the accomplishment of water movement up the stems of plants. Dixon proposed this theory to account for the apparent rise of water in the absence of evaporation. It is our opinion that the influence of saturation deficits was not properly obviated in this connection.

Bose's theory of a hydraulic pumping action of cells in the inner cortical regions of the stems finds no support whatever in this

work. There is no secretion of water from living cells in any case studied. The ascent of sap is due entirely to physical and chemical forces, if we can call osmotic action purely physical and chemical in nature. We prefer so to consider it. If evaporation is the only factor in the development of the forces of sap rise, how are these forces developed and applied to the columns of water in the plant? Some of the essential features of the development of water-lifting forces in the leaf colloids were discussed by one of the writers (8) several years ago. It may be profitable to review one or two features of the views previously set forth.

The loss of water from the plant is brought about solely by the kinetic activity of the water molecules themselves. The presence of gases around the plant is a hindrance to water loss, as evaporation occurs much more rapidly in a vacuum than at atmospheric pressure. More than any other gas, water vapor in the atmosphere tends to check water loss from the plant; and the rate of water evaporation is determined mainly by the relative humidity of the air. As this humidity decreases, the atmosphere becomes more and more nearly a water vacuum. Other factors, such as air temperature and barometric pressure, movements of the atmosphere, insolation, etc., influence the degree of water vacuum about the plant, and so modify the rate of water loss. Changes are also wrought in the internal mechanism which may modify the rate of water loss, such as the degree of opening and closing of stomata, changes in the permeability of cell membranes, incipient drying of internal walls, etc. In connection with sap ascent, the environmental situation is exceedingly important, and the degree of water vacuum is very important in determining the amount of pull which can be developed in the leaves.

Fig. 1 indicates diagrammatically the various forces involved in water loss, water retention, and water movement by arrows of different lengths, the magnitude of the force being relative to the length of the arrow. In section A the magnitude of the forces of evaporation is represented by an outwardly directed arrow, and the magnitude of the forces exerted by water molecules returning from the air to the colloidal tissues is represented by inwardly directed arrows. It must be evident that the rate of loss at any given moment is related to the difference between these two forces. When there is abundance

of free water in the leaf tissue, as at saturation, the outwardly directed arrow would have its maximum length. The difference between the two forces for loss and gain of water would then fluctuate with the length of the inwardly directed arrows (the magnitude of the forces of hygroscopic absorption of water). If the air is nearly saturated, the inwardly directed forces are nearly as great as the outwardly directed ones, and the difference effective for evaporation is small. But when the air is dry the inwardly directed forces are small, and the difference then effective for evaporation is large.

The main concern with reference to the forces of sap rise is what happens in the colloidal material when the tissue begins to lose water faster than it receives it. Then a saturation deficit develops, and the relation of the forces will be as represented in section *B* (fig. 1). With deficit, the outwardly directed forces become smaller and smaller, as indicated by the shorter arrows. Whenever the drying of the tissue reduces the outwardly directed forces to the same size as the inwardly directed ones (*A*), the system is in dynamic equilibrium, and no more water loss would occur; that is, loss and gain would be equal. This occurs rarely except with seeds or other dry organic matter, for in living plants the

Fig. 1.—Diagrammatic representation of forces of sap rise (outwardly directed arrow, force of evaporation; inwardly directed arrows, forces of atmospheric humidity): *A*, forces of evaporation and humidity; *B*, forces of evaporation, decreasing with development of saturation deficit; *C*, imbibitional force developed on individual particle, which serves as fulcrum for application of force to water movement; *p*, protoplasm; *w*, wall; *a*, internal atmosphere of leaf.

saturation deficit required to move water in the plant is small. It seems that a vapor pressure deficit of 2 per cent is probably more than enough to lift the sap to the top of the tallest living trees.

Just how is this lifting force created? The answer to this question is to be obtained from a consideration of the relation of the colloidal particles forming the surfaces where the saturation deficit is developing to the water present in connection with those particles. There is a mutual attraction between the molecules of water and the surfaces of the colloidal particles. This mutual attraction results largely from the presence of secondary valencies in the water molecule and in certain of the atoms occurring in the surfaces and interiors of the colloidal particles, particularly whenever the water deficit is pronounced. The water can evaporate from these surfaces only by overcoming all of the attractions occurring on and in the drying colloids.

If we consider the forces in connection with the individual colloidal particles of the membranes, the origin of the pull for water becomes clear. With water deficit, the drier the colloidal particle becomes, the larger is the force which it exerts for the retention of the remaining water. It exerts this force in every direction about it, as indicated in section C of fig. 1. This portion of the figure indicates that an individual surface particle exerts an attraction toward water molecules in the atmosphere, as well as toward those farther back in the tissues, away from the atmosphere. But as there is no continuity of material on the atmospheric side, the pull is actually being made effective only for the molecules of water that lie deeper in the tissue. As the surface pull enlarges, the deeper molecules of water, from wall, protoplasm, and vacuole, speedily move toward the surface in response to this colloidal pull. It seems clear that the solid substance of the wall acts as a fulcrum by means of which the forces of the saturation deficit can become effective in moving the water.

The forces developed in the colloids involve capillarity and surface tension, particularly in low-growing plants, in which the surface tensional forces of the menisci in the cell walls may be adequate for the entire phenomena of sap rise. But in taller trees, where the deficit is larger, the forces of adsorption are involved, including molecular, atomic, and ionic surface forces of the ultramicroscopic colloidal particles and water molecules. When the surface particles draw water from those next deeper to replace that lost by evaporation, those particles in turn replace their lost water from still deeper ones, and ultimately from the protoplasmic water, and from the vacuole of the cell. The removal of water from the vacuole by the

protoplast to relieve its own saturation deficit now concentrates the solutes in the cell sap, and converts the imbibitional or adsorption forces into osmotic forces. The osmotic forces are propagated from cell to cell, to the tracheal columns of water, from which the imbibitional and osmotic forces of the border parenchyma cells abstract water. In other words, in reality the saturation deficits and osmotic conditions mentioned merely provide conditions under which the water of the tracheae rapidly migrates into the leaf cells.

We believe that this development of attraction for water in the leaf colloids during evaporation is entirely adequate to account for the ascent of sap in all cases not involving root pressures. Whether evaporation is excessively rapid or extremely slow, the cause of sap movement is held to be due in any case to the development of surface tension, imbibitional and osmotic forces which are in no way related to excretory or secretory activity of the living cells.

Summary

1. These studies are concerned with the ultimate cause of the transpiration stream in the xylem tissue of vascular plants.

2. The current theories of sap rise involve active secretion of water by living cells of the leaves, excepting at times when the evaporation rate exceeds the secretion rate of these cells.

3. Repetitions of Dixon's experiments on sap rise with submerged shoots, and shoots transpiring into a saturated atmosphere without first satisfying the saturation deficit of the shoots, gave results in agreement with his findings. But in shoots in which the saturation deficit was first relieved there was no evidence of secretory activity of the leaf cells, and no significant rise of sap. The phenomena observed by Dixon are therefore believed to have been caused by saturation deficits in the twigs and shoots existing at the time the experiments were performed.

4. Although no experimental evidence is presented, it is believed that downward translocation of dye in the xylem, when leaves on wilty plants are truncated and supplied at the cut ends with eosin, is a consequence of root saturation deficit, which will be relieved from any direction by a supply of free water. It has nothing to do with the normal transpiration or translocation currents in plants.

5. Repetitions of Bose's bubbler experiments with transpiring

leaves also failed to show any evidence of protoplasmic secretion of water from the leaves. When leaves were coated with vaseline, after proper satisfaction of water deficit, transpiration ceased; and no water was found beneath the vaseline, even after 24 hours.

6. The data presented indicate that there is no reason for assuming any vital action in connection with theories of sap rise, except that of the osmotic action of living cells, in which osmosis ceases with death. Osmotic action is not considered vital action in the usual sense of that term.

7. Sap rise is believed to be caused by the surface tensional and imbibitional action of the cell wall substance, translated into osmotic action in the living cells.

8. A detailed discussion of the development of these forces in connection with the kinetic action of water, pressure and humidity of the atmosphere, the reduced vapor pressure of the leaf colloids, and the consequent pull of the surface forces of the colloids upon the deeper water of the cell is presented.

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REGENERATION IN LEAF CUTTINGS
OF IPOMOEA BATATAS

C. L. ISBELL

(WITH NINETEEN FIGURES)

Introduction

There is no general agreement regarding the exact meaning of the term regeneration as applied to plants, or how it differs from the usual methods of asexual propagation. KUPFER (5) believed the word ought to be limited to those cases in which an organ is formed at a place or under conditions in which it would not normally be formed. This seems to be as simple a definition as has been given, and explains, at least in a general way, how regeneration differs from asexual propagation.

Many have attempted to discover what there is in a detached part of a plant that gives it the power of regeneration. The early investigators in attempting to explain the phenomenon used parts of plants not normally producing shoots or roots. McCALLUM (6) stated that the leaves of many plants possess the power of organizing new shoot primordia. He showed a rooted begonia leaf and mentioned other plants that may be regenerated from leaves. VÖCHTING (9), cited by KUPFER (5), expressed the opinion that the leaf in which the power to regenerate is lacking would upon investigation prove to be the exception rather than the rule. KUPFER, however, experimented with leaf cuttings of 82 species and failed to confirm this. Roots were formed by 61 species while only 12 gave rise to shoots. She failed to obtain plants from leaf petiole cuttings of any species. She found that the base of the petiole of Irish potato leaf cuttings enlarged and served as a place of storage. BROUN (2), LOEB (4), and REED (7) worked with regeneration of *Bryophyllum* plants from leaves. LOEB's experiments tended to show that leaves of *Bryophyllum* would not produce roots and plants unless the leaf were detached or the transpiration stream between the leaf and other parts of the plant severed. BROUN showed that leaves of *Bryophyllum* would produce roots and shoots without their being detached or the trans-

piration stream being severed. REED made observations similar to
those of BROUN. He also found that the base of the petiole of de-
tached leaves of *Bryophyllum* rooted, and that the leaves with the
rooted petioles lived for several months without producing shoots.
He did not believe, like LOEB, that one part of a plant necessarily
inhibited the growth of other parts. He noted that the meristematic
tissue in the notches of the leaves of *Bryophyllum* where shoots arise
contained much pigment. CHILD and BELLAMY (3) showed that
under certain conditions a leaf of *Bryophyllum* might inhibit develop-
ment of a bud. The early investigators failed to reach an agreement
as to the cause of regeneration of roots and shoots from parts of
plants not normally producing these parts. They seemed to agree in
a general way, however, that leaf cuttings have more power to re-
generate roots than shoots.

More recently investigators have conducted experiments to deter-
mine the root and shoot responses from different kinds of shoot cut-
tings when placed under similar and under different environments.
The general conclusions from such researches are that the variety,
chemical composition of the cutting, presence or absence of foliage,
heat, light, moisture, or nutrients in the propagation medium or
treatment of the cutting with chemicals may influence the amount
and nature of the root and shoot response.

REID (8) found that shoot cuttings of the tomato sometimes
tended to do what the plant had been doing when the cuttings were
taken.[1] ZIMMERMAN and HITCHCOCK (10) observed that the leaf
petioles remaining on shoot cuttings of dahlias sometimes became
enlarged and served as a place of storage; moreover, the petioles of
leaf cuttings often rooted when placed in a propagation medium.
They found that shoot cuttings of dahlias taken at various seasons
showed peculiar varietal responses to changes in day length. They
also found that the length of day influenced the character of the root
system; short days encouraged storage roots and long days, fibrous
roots.

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[1] After this manuscript was prepared, a bulletin appeared (PRIESTLEY, J. H., and
SWINGLE, C. F., Vegetative propagation from the standpoint of plant anatomy. U.S.
Dept. Agric. Tech. Bull. 151), citing and discussing other literature treating of the effect
of the plant upon its regenerations.

Sweet potato plants are usually obtained by bedding the developed roots or by rooting vine cuttings. Plants can be produced by cutting sweet potatoes into pieces and planting somewhat as Irish potatoes. Plants are sometimes obtained from seed in hot climates, where there is a long growing season. KUPFER found that thin cross-sections of peeled sweet potato roots, when planted in sand, callused on the cut surfaces and regenerated roots from near the center on both surfaces. She also planted sweet potato peelings from which buds had been removed and obtained roots; during the two months of the experiment, however, none of the parts in which shoot buds were lacking regenerated buds. BEALS (1) removed one end of an enlarged sweet potato root by a transverse cut and found that a shoot regenerated out of the cambium at the cut surface.

The writer has not found any literature which refers to the regeneration of roots or shoots by sweet potato leaves. In June 1929 a few sweet potato leaves were placed in a propagation bed and roots began to develop within 3 days. This immediately raised the question as to what part or parts of the leaf would regenerate roots. It also raised the question whether a new plant would arise from the leaf cutting, and, if so, what would be the location of the adventitious bud which produced the new shoot. Different types of sweet potato leaf cuttings were taken, rooted, and observed until plants or potatoes or both were produced. Observations made on these cuttings are reported in this paper.

Procedure

Cuttings were taken on bright sunny days not earlier than noon, usually not until late afternoon. Cuttings were taken at this time because of the general belief that the leaf is more likely to contain a large amount of carbohydrate material after it has been exposed to light for some time, and that soft wood cuttings root better when sufficient carbohydrate material is present.

Cuttings from four different varieties were observed. The pigmentation other than that due to chlorophyll (pigment in this paper does not refer to chlorophyll) varied in different varieties. At least three varieties were included in some of the types of cuttings studied. There were seven general types of cuttings observed; some of them

of one type were further modified when transferred from the propagation bed. The types of cuttings are described as follows:

1. An entire leaf carefully removed from the vine so that no part of the stem was left on the petiole. Some of these cuttings were further modified by the removal of the leaf blade at the time the cuttings were potted.

2. A leaf blade and half of the leaf petiole.

3. A leaf with the blade and petiole reduced one-half.

4. A leaf blade and a portion of the petiole, prepared by removing all of the petiole except the enlarged portion where it united with the leaf blade.

5. Like type 4, except that the entire petiole and the thickened part of the leaf blade where the leaf and petiole unite were removed, the remainder of the leaf blade being used as the cutting.

6. Terminal one-half of the midrib and one-fourth of the leaf blade, prepared by cutting a leaf blade transversely into two parts of about equal leaf surface. The terminal half of the leaf blade was used as the cutting, after it was further modified by removing enough of the leaf surface to allow the basal part of the midrib to extend into the sand.

7. Leaf petiole.

Figs. 1–6 illustrate types of cuttings 1–6 respectively, as they appeared when taken from the propagation bed and prepared for potting.

The cuttings were moistened with water as soon as taken, carried to the greenhouse, and placed in sand to root. The propagation bed was equipped with a frame and glass cover which were used to control the humidity. For the first 2 or 3 days after taking, the cuttings were sprinkled with water three or four times during the day to maintain a rather high humidity. Very frequent sprinkling was not practiced after that time. The glass cover was removed at night to allow ventilation.

Some of the cuttings were ready to be taken out of the sand in 5 or 6 days, but in general cuttings were left in the bed until practically all that were made at any given date were well rooted, which required about 10 days. The rooted cuttings were transferred to small clay pots containing potting soil. (At this time it was not expected

that the experiment would be continued beyond the time when a shoot appeared, therefore the pots containing the rooted cuttings were set in available greenhouse space without special regard to light,
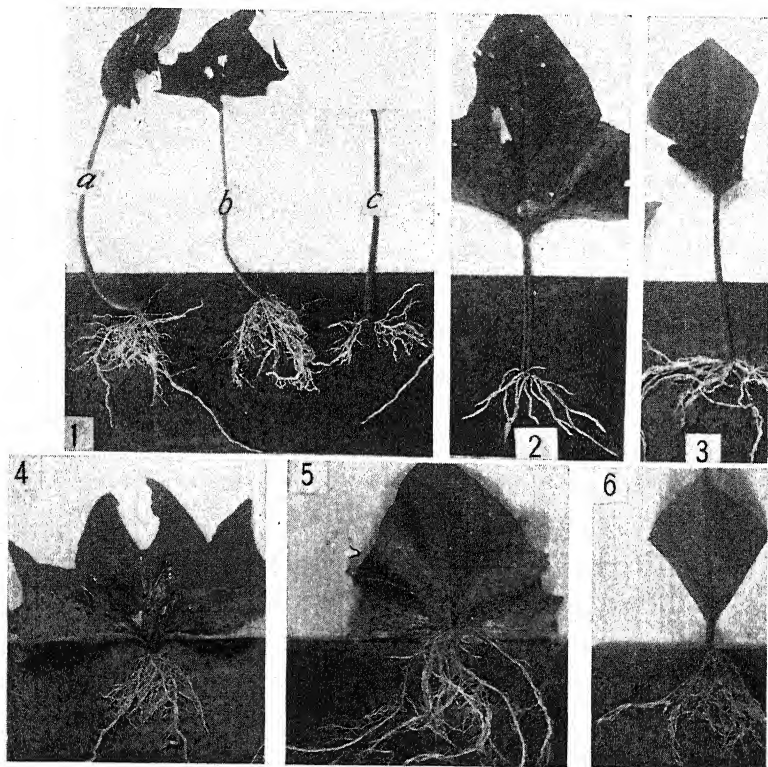
FIGS. 1–6.—Fig. 1, representative cuttings of entire leaves: *a*, non-pigmented; *b*, pigmented; *c*, leaf blade and petiole with leaf blade removed at time cutting was potted. Fig. 2, leaf blade and half of petiole. Fig. 3, reduced leaf blade and half of petiole. Fig. 4, reduced leaf blade and enlarged part of petiole where it unites with leaf blade. Fig. 5, leaf blade with part of base removed. Fig. 6, portion of leaf blade and part of leaf midrib.

heat, moisture, or ventilation. Actually, the plants were in shade most of the time.) Some were transplanted from the pots to the field. At the end of the experiment the older cuttings and their regenerations were growing in 8-inch pots, and the younger ones, or those taken last, were in 4-inch pots. About a month before the experiment was dis-

continued all pots were transferred to the open, and the cuttings left in them. Cuttings which were potted last were not repotted, unless taken up to photograph.

Results

Observations were made at frequent intervals during the progress of the experiment, and all plants were examined when the experiment was being discontinued October 3 and 4. Results obtained from pot-grown plants are shown in table I. It will be seen that all of the cuttings of type 1 (which included varieties with a wide range in pigmentation) had not produced shoots when the experiment was discontinued. Most of the cuttings in this group failed to regenerate shoots from fibrous roots. Fig. 1 illustrates some of the cuttings of this type, photographed after they had been in the propagation bed 12 days. It will be seen that all cuttings did not behave alike. One kind of response, characteristic of the variety that contained no pigment, was for the leaf blade to die before a shoot was regenerated (fig. 1a) or soon after (fig. 9c), or to remain alive while the lower part of the petiole enlarged (fig. 11). One cutting with enlarged petiole was kept under observation after the others had been discontinued; it finally produced a shoot from the callus of the petiole. Another kind of response, typical of a variety with some pigment, was for the cutting to produce a potato and to form shoots from the callus on the enlarged base of the petiole and from the potato at about the same time. A cutting of this type is shown in fig. 7, with a shoot from the callus and from the potato at b and c respectively. The variety with much pigment in the leaves tended to form shoots rather quickly from the callus, and in some instances the parent leaf remained alive until its shoots had made considerable growth. There was a general tendency for cuttings of this variety to produce more than one shoot from the callus on the petiole. Fig. 8a illustrates cuttings of this variety with a shoot (b) just as it was developing from the callus 18 days after the cutting was taken. The same parent leaf is shown in fig. 9a as it appeared 18 days later, after two other shoots had appeared. This leaf was alive when its shoots had reached 48, 14, and 6 inches in length respectively.

In the cutting of type 1 (fig. 1c) the leaf blade was removed at the time of potting, to determine whether the rooted petiole could re-

TABLE I

GROWTH OF DIFFERENT TYPES OF SWEET POTATO LEAF CUTTINGS

TYPE OF CUTTING	NUMBER	DATE CUT (1929)	DATE OF FIRST SHOOT (1929)	NO. PRODUCING SHOOTS	AVERAGE NO. SHOOTS PER CUTTING	ORIGIN OF SHOOT*				CHARACTER OF ROOT SYSTEM			CONDITION OF PARENT LEAF	
						No. from callus	No. from between callus and potatoes	No. from potatoes	No. from small or fibrous roots	No. producing small or fibrous roots	No. producing enlarged or stringy roots	No. producing potatoes	No. dead	No. fair to good condition
1 (Entire leaves): Pigment ranging from much to none........	21 11	6/21 7/6	7/21 9/18	17 2	1.20 0.18	16 0	4 0	5 0	0 2	2 5	8 6	11 0	14 0	7 11
Pigment in few leaves........ Leaf blade removed when potted; some pigment........	2	6/21	7/3	1	0.50	1	0	0	0	1	1	0	0	2
2 (Leaves with 1/2 petiole removed when cuttings taken): No pigment........ No pigment........	11 2	6/30 7/6		0 0	0.00 0.00	0 0	0 0	0 0	0 0	9 2	0 0	2 0	0 0	11 2
3 (Leaves with blades and petioles reduced at cutting): Some pigment with blades and petioles reduced	10	7/6	9/8	6	1.00	2	0	8	0	2	8	0	0	10
Some pigment in all cuttings........	13	6/30	8/23	5	0.62	2	0	0	7	10	3	0	1	12
4 (Leaf blades with petiole removed except enlarged part where petiole unites): All leaves had much pigment........ All leaves had much pigment........	12 12	6/30 7/6	8/3 8/30	12 9	1.33 0.83	13 6	0 0	2 2	1 1	5 2	5 8	2 2	3 2	9 10
5 (Leaf blades with entire petiole removed including enlargement where petiole unites): All leaves including pigment........	6	8/3	9/30	2	0.33	1	0	1	0	0	5	1	0	6
6 (Small part of leaf blades from terminal part of leaves with part of midrib): Leaves containing some pigment........	10	8/3	10/4	1	0.10	0	0	0	1	9	0	1	0	10
7 Leaf petioles........	10	6/21	Cuttings of this type observed only until roots formed.											

* In several instances it was almost impossible to ascertain whether shoot originated from callus or potato or some point between.
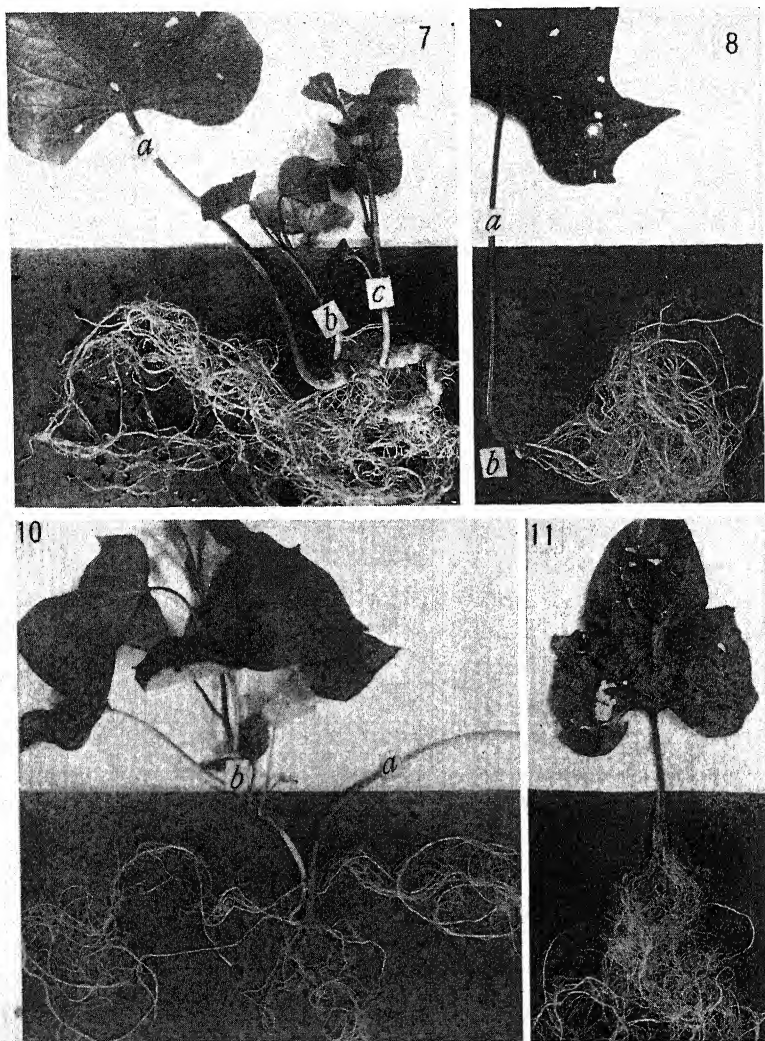
FIGS. 7, 8, 10, 11.—Fig. 7, *a*, parent leaf of variety containing some pigment; *b*, shoot arising from enlarged callus; *c*, shoot arising from potato. Fig. 8, *a*, parent leaf of variety containing much pigment; *b*, young shoot appearing from callus. Fig. 10, *a*, parent petiole from which entire leaf blade was removed on potting; *b*, shoot regenerated from callus on base of petiole. Fig. 11, leaf blade and half of petiole, showing base of reduced petiole enlarged and serving as place of storage.

generate a shoot with this removal. From table I it will be seen that one of the two cuttings of this type produced a shoot and the other did not. Fig. 10 illustrates the cutting with its shoot as it appeared 68 days after the cutting was made. When examined October 3, the cutting which did not produce a shoot apparently had changed very little from the time it was potted, except that the older roots appeared to be breaking down and new ones forming. The parent leaf petiole was still alive and appeared healthy. The cuttings of type 1

grown in the open be-
haved much as did those
in pots, except that the
shoots which they regen-
erated tended to make
more vine growth. The
length of shoots of one of
these cuttings totaled 50
feet of vine when the ex-
periment was discontin-
ued.

It will be noted from
table I that cuttings of

FIG. 9.—*a*, parent leaf (same as fig. 8) after three shoots had appeared; *b*, parent leaf of variety producing relatively few shoots; *c*, parent leaf that died soon after shoot appeared.

type 2, from the variety without pigment, had not produced shoots when the experiment was discontinued. All of the non-pigmented leaves were alive at this time. The petioles of some of the cuttings of this type split in the propagation bed; such petioles healed and served as a place of storage. The petioles that did not split also enlarged and served for storage (fig. 11). Cuttings of type 2 from a variety with some pigment tended either to produce potatoes from which shoots developed rather quickly (fig. 12), or to produce a potato directly from the petiole and then to form a shoot (fig. 13). Cuttings of the variety with heavy pigment produced plants rather quickly and freely from the callus of the petiole (fig. 14). The basal portion of the petiole of this variety did not tend to enlarge and serve as a place of storage.

Seven out of nine of the shoots produced by cuttings of type 3 originated from fibrous roots, and the other two from the callus. There was little or no tendency for the reduced petiole to enlarge and serve as a place of storage, or for potatoes to be formed before shoots.

The variety represented in this type was not represented in type 2, which may account for the difference in behavior of the cuttings of types 2 and 3. This type of cutting is illustrated in fig. 15, showing at *b* a shoot arising from a fibrous root which came to the surface. Fig. 15 also shows a young shoot developing at *c* on the same root nearer the parent leaf. A younger shoot is developing at *d* from another fibrous root of the same plant.
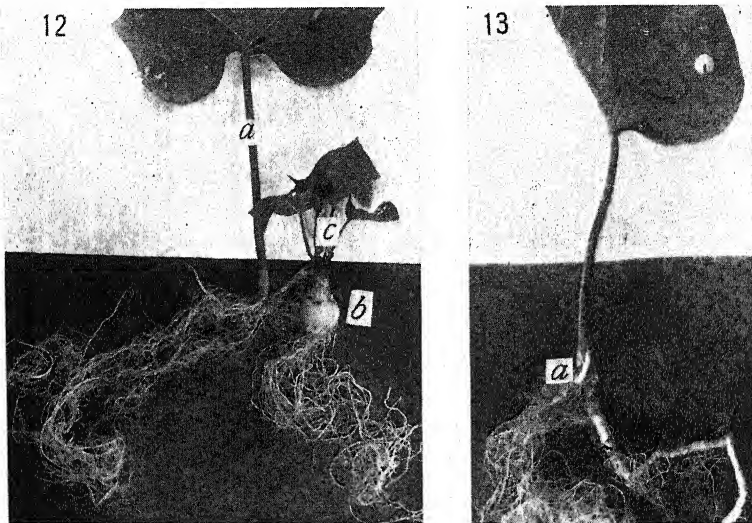
FIGS. 12, 13.—Fig. 12, *a*, leaf blade and half of leaf petiole; *b*, potato; *c*, shoot produced from it (photograph taken 2 months, 8 days after cutting; potato weighed 17 gm., green weight). Fig. 13, same behavior as fig. 12, except that potato was produced directly from end of reduced petiole; *a* arose from point near where potato grew out.

Type 4 gave the highest number of shoots per cutting. Nineteen of the 25 shoots produced by cuttings of type 4 arose from the callus (figs. 16, 17), two from fibrous roots (fig. 17*c*), and four from potatoes. Table I shows that relatively few of the cuttings of type 5 had produced shoots when the experiment was discontinued. This can probably be explained by the cuttings being taken later than the others. A cutting of this type is shown in fig. 18 with a shoot (*b*) arising from the callus. A cutting of the same type is shown in fig. 19, with the leaf blade partially torn apart to show a greater root

development from the midrib than from smaller veins. A potato was developed on the midrib. The potato produced a shoot.

One cutting of type 6 produced a potato directly on or out of
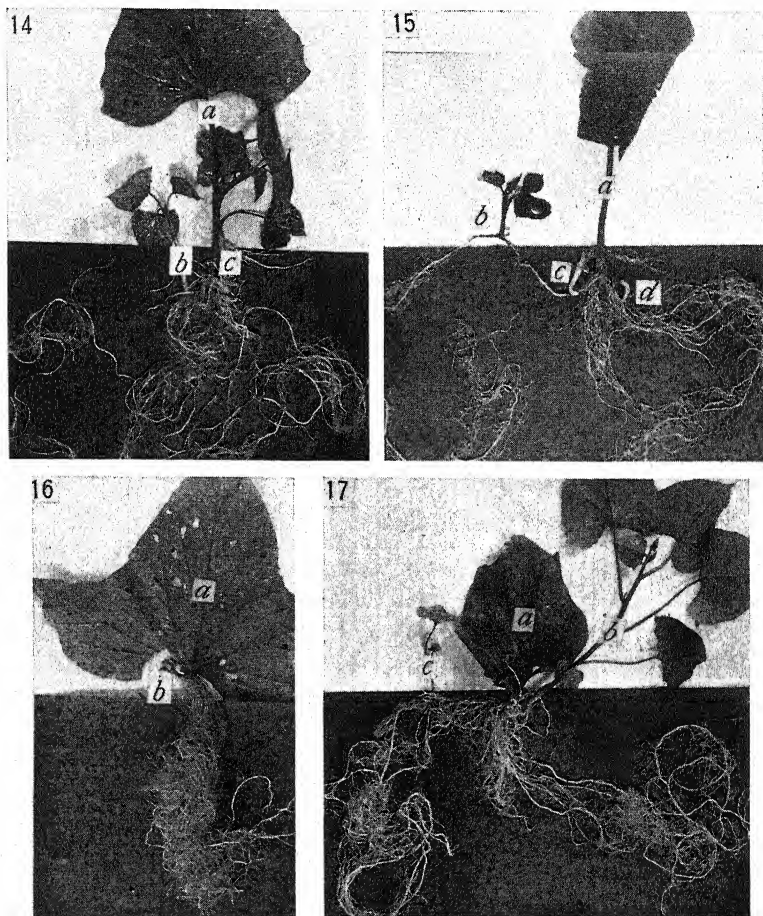
FIGS. 14–17.—Fig. 14, *a*, parent leaf of variety containing much pigment; *b, c*, shoots arising from base of reduced petiole. Fig. 15, *a*, parent leaf with reduced leaf blade area and reduced leaf petiole; *b*, shoot regenerated from fibrous root that came to surface; *c*, shoot regenerated from same fibrous root as *b; d*, young shoot developing on another fibrous root. Fig. 16, *a*, leaf blade and terminal or enlarged part of petiole; *b*, shoot regenerated from callus. Fig. 17, *a*, same type as *a* of fig. 16; *b*, shoot arising from callus; *c*, shoot arising from small root.

the base of the reduced midrib. A cutting of this type regenerated a shoot out of the base of the reduced midrib. Cuttings of type 6 were used to determine how small a leaf area might be used to regenerate roots or plants.

Some of the cuttings of type 7 rooted, but were discarded soon after they were removed from the propagation bed.
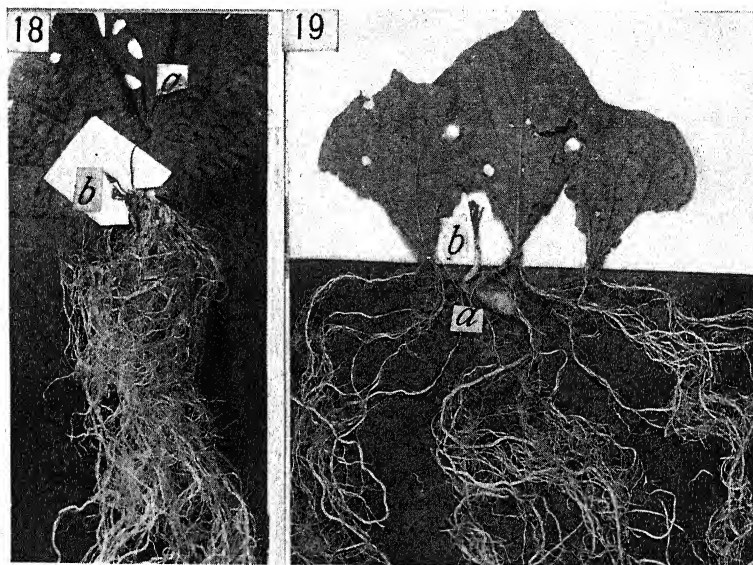
FIGS. 18, 19.—Fig. 18, *a*, leaf from which petiole and part of leaf blade were removed on cutting; *b*, origin of shoot. Fig. 19, same type as fig. 18; parent leaves partially torn apart to show development of roots from different regions: *a*, potato; *b*, shoot.

Discussion

Sweet potato leaf cuttings from different varieties behaved differently. It cannot be stated from the data whether the behavior of different cuttings was due to variety, to amount of pigment in the cutting, or to some other cause. The effect of the stage of growth of the plant from which cuttings were taken on the behavior of the cuttings, as reported by REID (8) for tomatoes, was not ascertained. REED (7) kept leaves of *Bryophyllum* with rooted petioles in sand for 6 months without the production of shoots: if the rooted petioles enlarged and served as a place of storage as did some of the sweet

potato petioles he failed to mention it. The failure of rooted leaves of *Bryophyllum* to regenerate shoots was used by REED (7) to question certain of LOEB's (4) theories of the inhibitory effects of one part on another. The behavior of cuttings of different varieties of sweet potato leaves suggests that this plant might be of value for work such as LOEB carried on with *Bryophyllum*. A comparative study of varieties using the petiole as a place of storage and those that early differentiate a bud from the petiole might have more nearly harmonized the ideas of LOEB and REED concerning the inhibition and regeneration of shoots from leaf cuttings.

The petiole which regenerated a shoot after the entire leaf blade was removed (fig. 10) shows that rooted petioles of that variety are not necessarily dependent on leaf blades for power to regenerate a shoot. KUPFER (5) failed to find any species in which an isolated petiole formed a shoot. In the work reported in this paper petioles without an attached leaf blade formed a rather limited root system, but were not observed long enough to determine whether they would develop shoots.

In type 3 the appearance of shoots on small roots at different distances from the parent leaf, as well as the appearance of more than one shoot on a small root, is interesting in connection with the statement by CHILD and BELLAMY (3) that a leaf may inhibit a bud and that one part may inhibit another within certain distances. It is also of interest to note that enlargement of the petiole was not found with this group of shoots. The appearance of the first shoot from a root that came to the surface is of interest in connection with the fact that REED (7) found that the *Bryophyllum* leaf produced plants much sooner in the dark.

Under the conditions of this experiment, there appeared to be a quicker shoot response from pigmented cuttings. There also appeared to be a quicker shoot response from leaf cuttings with reduced or no petioles than from those with entire petioles. It is suggested that the cuttings with more petiole had the power to use the petiole for storage, and in this way tended to inhibit shoot formation.

Since there appears to be a rather definite relationship between root development from the midrib and that from other veins (fig. 19), it is suggested that this type of cutting might be used to measure

the amount of material manufactured by the different parts of the leaf blade.

Summary

1. Six different types of sweet potato leaf cuttings were rooted and observed until plants were regenerated. A seventh type was discarded after roots regenerated but before shoots were formed.

2. All types of cuttings regenerated roots quickly.

3. When the leaf blade was removed from a rooted leaf cutting the remaining petiole had the power to regenerate a shoot.

4. Leaf blades or parts of leaf blades with or without the petiole regenerated roots and shoots.

5. Cuttings regenerated shoots from the callus on the petiole, from fibrous roots, from fleshy roots, or from more than one of these points.

6. Leaf blade cuttings produced most of the roots out of the midrib and veins, the former showing the greater development.

7. In some cuttings potatoes were produced directly on or out of the petiole or reduced midrib.

8. Entire petioles or reduced petioles of cuttings from a non-pigmented variety enlarged and served as a place of storage. These failed to differentiate shoots or did so very slowly.

9. Leaf cuttings of a variety with a small amount of pigment tended either to produce a potato before a shoot differentiated or to differentiate shoots from fibrous roots.

10. Leaf cuttings of a heavily pigmented variety produced shoots rather freely either from the callus on the petiole or from small roots.

ALABAMA EXPERIMENT STATION
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FLORAL MORPHOLOGY OF LYONOTHAMNUS FLORIBUNDUS

JOSÉ B. JULIANO

(WITH TWENTY-NINE FIGURES)

Introduction

There is a conflict of opinion as to the systematic position of *Lyonothamnus floribundus* Gray, a flowering tree endemic to the Channel Islands off the coast of California. GRAY (8), as well as GREENE (9) and JEPSON (14), believed that despite its saxifragaceous fruit it should be placed in the Rosaceae. SARGENT (22) at first considered it one of the Saxifragaceae, but later (23) accepted GRAY's opinion. BRITTON (2) removed it from the Saxifragaceae and put it under the closely allied family of Cunoniaceae. This investigation was undertaken at the suggestion of Professor LEROY ABRAMS, in the hope that the internal morphology of the flower might shed light on these conflicting views.

The material used was gathered at frequent intervals, from May 16 to August 16, 1929, from two healthy trees, one of which grows in the garden of Professor DURAND on the Stanford University campus, the other on the campus of the University of California.[1] A chromoacetic solution, prepared according to the formula given by CHAMBERLAIN (3), Bouin's fluid (aqueous and alcoholic), and formalin-acetic-alcohol were employed as fixatives. The material was imbedded in the usual way, and sections were cut 10–15 μ in thickness. Most of the preparations were stained with Haidenhain's iron-haematoxylin and counterstained with aniline orange gold dissolved in clove oil. Flemming's triple stain and safranin-light green combination were utilized to advantage.

Investigation

FLOWER

The perfect small white flower, which is borne in a much branched and rather broad terminal panicle, arises from the ultimate branches

of the inflorescence. Each flower is subtended by two or three, sometimes four, small and acute persistent bractlets (fig. 1). The five sepals, which are inserted on a circular 5-lobed hypanthium, are nearly triangular, apiculate, and also persistent. The hypanthium is tomentose on its outer surface, and woolly hairs which persist to maturity of the fruit arise from the inner surface. JEPSON (14) mentions the deciduous character of the calyx lobes of *Lyonothamnus*, but my study indicates that they remain on the mature fruit, where they become rather brittle. On this hypanthium are five petals which alternate with the calyx lobes. The petals are nearly always orbicular, sessile, and white. Within the corolla is an outer whorl of ten stamens, one opposite each petal and sepal, and an inner whorl of five stamens, one opposite each sepal. The number of stamens is irregular, however, and varies from thirteen to sixteen. Inclosed by the hemispherical hypanthium are two pistils, each bearing a rather club-shaped style and capitate stigma, which arise from the abbreviated floral axis (receptacle). Three pistils are sometimes present. The ovaries are mono-carpellary and usually contain four ovules, although six are not uncommon. The anatropous oblong ovules, which are suspended on long funiculi, possess superior micropyles at their maturity, and are borne one above the other on the ventral parietal placenta in two distinct series.

The individual flower starts as an emergence from the axil of a bract. Usually three floral primordia arise simultaneously (fig. 3), the middle one always developing much more rapidly than the lateral ones. The first members to differentiate are the bractlets, which arise as lateral mammillate protrusions (fig. 2). These soon elongate and cover the floral axis (fig. 4). As soon as the bractlets are well differentiated the five sepals develop simultaneously from the periphery of the receptacle. Within the calyx whorl and alternating with the young sepals, the five petals next appear simultaneously as mammillate humps (fig. 5). There follows an elongation of the tissue on which the sepals and petals are borne. Because of the elongation of the hypanthium, the apex of the floral axis becomes a depression within it.

After the hypanthium has elongated somewhat, the primordia of the outer whorl of stamens appear, one opposite each petal and sepal,
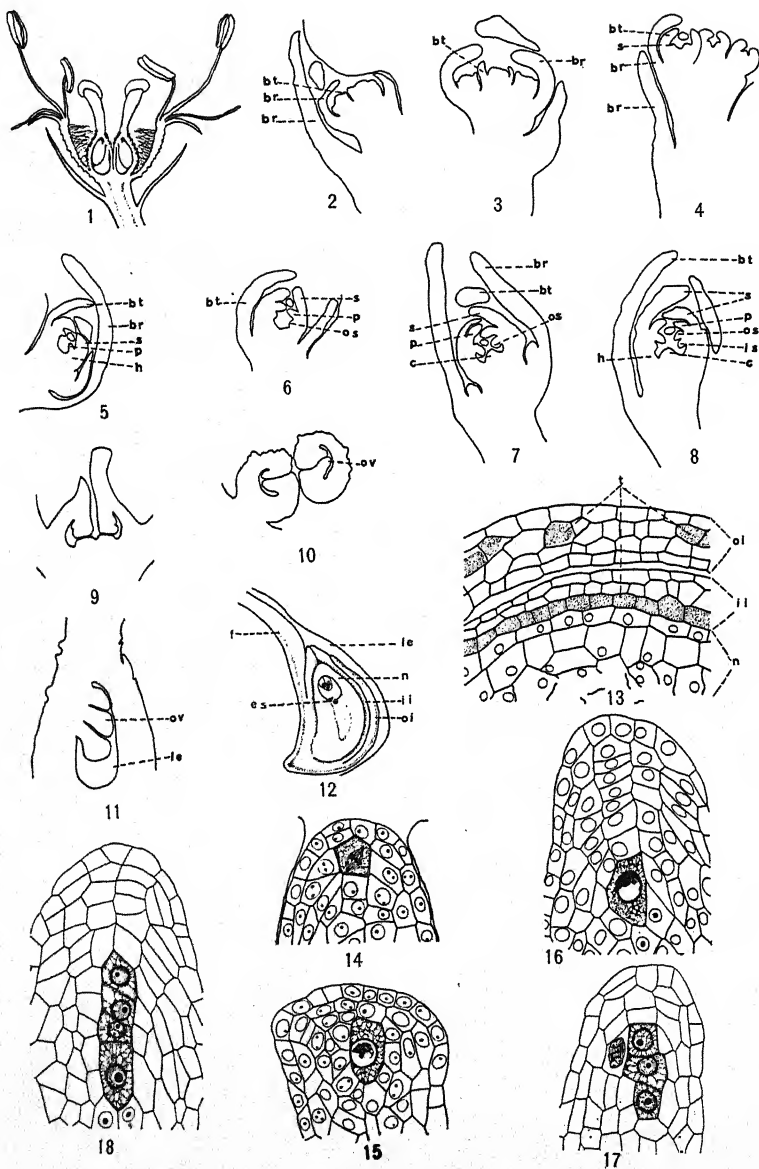
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just within the petals (fig. 6). As the outer whorl of stamens starts to lengthen, there begins the development of two, sometimes three, hemispherical carpellary humps (fig. 7) from the apex of the receptacle. The inner whorl of five stamens, one opposite each sepal (fig. 8), develops last. In other words, development of the floral organs in *Lyonothamnus* is not acropetal, and they arise as follows: bractlets, sepals, petals, outer stamens, carpels, and inner stamens. COULTER and CHAMBERLAIN (4) cite that HOFMEISTER records the development of the carpels in *Rosa*, *Potentilla*, and *Rubus* before those of the stamens have reached their full number, a feature similar to that existing in *Lyonothamnus*. On the other hand, in *Astilbe japonica* WEBB (30) finds a similar development of the carpels before the last whorl of stamens is formed, but in this plant the outer whorl of stamens is the last to be developed. The sequence of appearance of the floral parts, therefore, is not a good criterion for determining whether *Lyonothamnus* should belong to the Rosaceae or to the Saxifragaceae.

FIGS. 1–18.*—Fig. 1, longitudinal section of mature flower. Fig. 2, portion of median section of branch of inflorescence showing floral primordia at axil of bract; bractlets beginning to differentiate. Fig. 3, older branch showing floral primordia with bractlets already differentiated. Fig. 4, much older branch showing differentiation of sepals in floral primordia. Fig. 5, longitudinal section of single flower with bract, bractlets, sepals and petals (note growth of hypanthium). Fig. 6, longitudinal section of older flower showing beginnings of primordia of outer whorl of stamens. Fig. 7, longitudinal section of much older flower showing beginnings of carpel. Fig. 8, longitudinal section of flower after differentiation of parts. Fig. 9, longitudinal section of pistils showing origin of ovules. Fig. 10, transverse section of ovaries showing two series of ovules. Fig. 11, longitudinal section of older ovary showing three ovules in single series. Fig. 12, diagram of longitudinal section of mature ovule showing relative lengths of megagametophyte before and after absorption of chalazal nucellar tissue; two antipodal cells (blackened) still persisting. Fig. 13, portion of longitudinal section of integuments and nucellus of mature ovule. Fig. 14, nucellus with archesporial cell dividing. Fig. 15, nucellus after formation of primary parietal cell and megaspore mother cell. Fig. 16, deep-seated megaspore mother cell in synapsis (note two conspicuous nucellar cells below spore mother cell). Fig. 17, megaspore mother cell and two lower nucellar cells exhibiting same staining capacity, size of nuclei, and amount of cytoplasm. Fig. 18, linear tetrad of four megaspores; chalazal megaspore enlarging; two basal nucellar cells well differentiated.

* Designations are as follows: antipodals *an*, basal apparatus *ap*, bract *br*, bractlets *bt*, carpels *c*, cotyledon *ct*, embryo *em*, endosperm *en*, hypanthium *h*, hypostase *hy*, inner integument *ii*, inner stamens *is*, locule *le*, megagamete *e*, megagametophyte *es*, nucellus *n*, outer integument *oi*, outer stamens *os*, ovule *ov*, petals *p*, plumule *pl*, polar nuclei *pn*, pollen tube *pt*, radicle *r*, raphe *re*, tanniferous cells *t*, seed coat *te*, vascular bundle *vb*, and zygote *ze*.

MEGASPORANGIUM

The development of the megasporangium has been traced and shows nothing novel. Four (figs. 9, 10), sometimes six (fig. 11), ovules emerge as mammillate protuberances on the parietal placentae, and these soon elongate laterally and sometimes obliquely downward into the loculus of the ovary, forming the nucellus of the nascent ovules. Integuments are developed in basipetal succession. At maturity of the ovule the outer integument reaches only a little beyond the level of the apex of the nucellus (fig. 12), and consists of four layers of cells (fig. 13). Underneath its outer epidermis is a layer of tanniferous cells. The inner integument completely covers the nucellus and consists of three layers of cells. Toward the micropyle the hypodermal cells of the inner integument divide longitudinally and an increase in breadth results. The innermost layer of cells of the inner integument possesses tanniferous substance, and this layer delimits the nucellus to the chalaza.

An obturator is formed by a great number of the rosaceous genera, especially those belonging to the Spiraeoideae, Pomoideae, and Prunoideae (13). This obturator, which develops simultaneously with the ovules, is morphologically considered by PÉCHOUTRE (20) to be an abortive ovule, and on this assumption he explains its absence in most if not all of the "Spirées." This structure is unknown in the Saxifragaceae, except for *Francoa appendiculata* in which GÄUMANN (7) reports the development of a "Funiculuswarze" which functions as an obturator. No obturator is formed in *Lyonothamnus*, which produces a variable number of ovules. It is interesting to note, however, that at the time the megaspore mother cell is formed, there is almost always a faint differentiation of a few cells of the funiculus of the uppermost ovule just close to the ovarial wall. A few of the epidermal cells become palisade-like and stand out conspicuously from the rest of the cells. This differentiation is so slight that one is apt to overlook it. Aside from this feeble growth of a few funicular cells of the uppermost ovule in each series, there is nothing suggesting an obturator in *Lyonothamnus*. In this respect the ovule is saxifragaceous rather than rosaceous in nature.

As soon as the inner and outer integuments have been fully differentiated, and long before the micropyle becomes superior owing to

the growth of the funiculus, there is the differentiation of a one-celled archesporium (fig. 14). This archesporial cell divides periclinally, giving rise to a primary parietal cell and a megaspore mother cell (fig. 15). The development of a many-celled archesporium, a character true to a majority of the Rosaceae (13), has not been observed in *Lyonothamnus*.[2] Among the Saxifragaceae there is generally found a development of a one-celled archesporium: *Saxifraga* (15, 19, 24), *Heuchera* (19), *Chrysosplenium* (15, 28), and *Ribes* (6, 11). The archesporium may function directly as a megaspore mother cell in *Parnassia palustris* (19), and no parietal tissue is consequently developed. Exceptions to this are to be seen in the many-celled archesporium of *Astilbe japonica* (30), *Philadelphus* (7), and *Ribes nigrum* (29). On the whole one may say that a several-celled archesporium is generally found in the Rosaceae and a single-celled archesporium in the Saxifragaceae. That of *Lyonothamnus*, therefore, is saxifragaceous rather than rosaceous in nature.

Considerable parietal tissue is developed while the megaspore mother cell is in synapsis (fig. 16), so that the megaspore mother cell comes to lie deeply sunken in the nucellus. Among the Rosaceae, PÉCHOUTRE (20) finds the formation of two to several layers of parietal tissue, and this is accompanied by periclinal divisions of the nucellar epidermis which forms a covering (the "coiffe épidermique") over the latter. In the Saxifragaceae some genera have the development of a rather thick parietal tissue, while others produce none at all. The Saxifragaceae never form a "coiffe épidermique." *Lyono-*

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[2] A survey of the literature on this question shows that multicellular and unicellular archesporia are formed as follows in the two families:

ROSACEAE
    Multicellular archesporium:
      Pomoideae: *Eriobotrya, Pirus, Malus, Amelanchier* (13).
      Rosoideae: *Kerria, Rhodotypus, Rubus, Fragaria, Dryas, Geum, Alchemilla, Sanguisorba, Agrimonia, Rosa* (13), and *Potentilla* (24).
      Prunoideae: *Prunus* (13).
    Unicellular archesporium:
      Neurada (18), *Amygdalus campestris* (15), *Prunus* (21).
SAXIFRAGACEAE
    Multicellular archesporium:
      Astilbe japonica (30), *Philadelphus* (7), *Ribes* (29).
    Unicellular archesporium:
      Saxifraga (15, 19, 24), *Heuchera* (19), *Chrysosplenium* (15, 28), and *Ribes* (6, 11).

thamnus, which develops a rather thick parietal tissue without an epidermal cap, has therefore a nucellus that is more saxifragaceous than rosaceous in nature.

Although a single megaspore mother cell is developed in *Lyonothamnus*, one usually finds some of the nucellar cells adjacent to the megaspore mother cell exhibiting characters typical of the latter. In fig. 17, for example, just beneath the megaspore mother cell are two nucellar cells which exhibit the same staining capacity, size of nuclei, and amount of cytoplasm. To the left is another nucellar cell undergoing vegetative division. These conspicuous nucellar cells never give any indication of functioning as spore mother cells, although they may remain evident up to the binucleate stage of the megagametophyte. Although *Lyonothamnus* develops a saxifragaceous archesporium, this differentiation of several of its nucellar cells may suggest that it is not far removed from the Rosaceae, and that it may have come from a rosaceous ancestor which develops many megaspore mother cells either from a single or multicellular archesporium.

MEGAGAMETOPHYTE

By two successive divisions the megaspore mother cell gives rise to a linear tetrad of four megaspores (fig. 18), the chalazal, the third megaspore of which rarely becomes functional. A normal 7-celled megagametophyte is formed by three successive divisions of the nucleus of the functional megaspore. In the mature megagametophyte (fig. 19) the egg apparatus (megagamete and two synergids) and two polar nuclei occupy its micropylar end. The megagamete usually lies between the synergids. At the chalaza the three antipodal cells are formed. These persist for some time after their formation and have a dense cytoplasm and distinct nuclei. They disappear during elongation of the megagametophyte and before fertilization.

Soon after the formation of the 7-celled megagametophyte, there is rapid destruction of the nucellar cells at the chalazal end (fig. 12). Accompanying this absorption of the chalazal tissue is an elongation of the gametophyte, which assumes a cylindrical or tapering shape with its swollen end directed toward the micropyle. As a result of this elongation the antipodals come to lie to one side before they
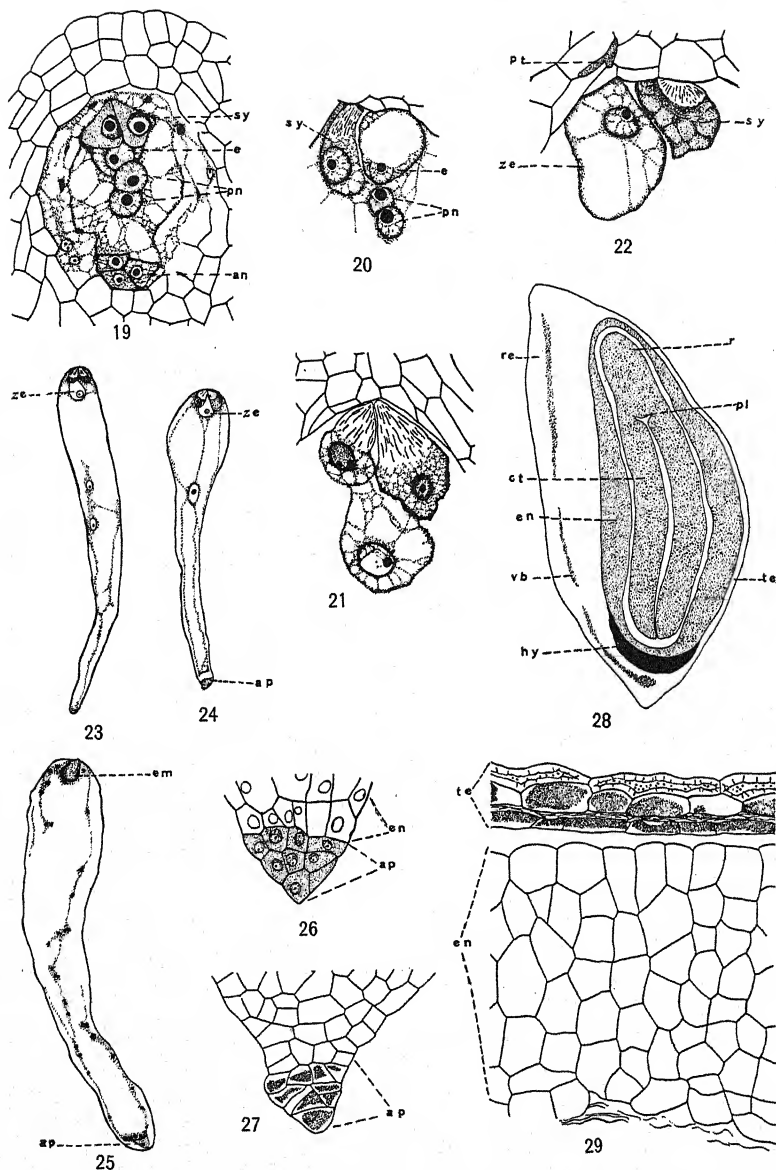
FIGS. 19–29.—Fig. 19, megagametophyte showing migration of chalazal polar nucleus and cells cutting off. Fig. 20, egg apparatus and polar nuclei; one of synergids not shown (note filiform apparatus at base of synergid). Fig. 21, egg apparatus before fertilization. Fig. 22, zygote. Fig. 23, longitudinal section of embryo sac showing zygote and first division of endosperm nucleus. Fig. 24, after small basal cell cut from primary endosperm cell. Fig. 25, free nuclear divisions in upper derivative from primary endosperm cell; basal apparatus still unicellular. Fig. 26, chalazal portion of endosperm showing multicellular basal apparatus. Fig. 27, degeneration of basal apparatus. Fig. 28, diagram of longitudinal section of mature seed. Fig. 29, portion of longitudinal section of mature seed showing seed coat and endosperm.

finally disappear. Nucellar cells persisting at the chalaza greatly elongate, while those next to the inner integument develop into a hypostase. This hypostase, a structure of universal occurrence among the Rosaceae and found in some of the Saxifragaceae, has become fully differentiated by the time the gametophyte has 8 nuclei.

Simultaneously with elongation of the megagametophyte further differentiation of the egg apparatus takes place. At the beaked extremities of the synergids are delicate longitudinal striations, the filiform apparatus (figs. 20, 21), occupying nearly half the length of the synergids. These structures stand out clearly when stained with safranin-light green. The filiform apparatus stains green while the distal cytoplasm of the synergids stains a deep red. This filiform apparatus exhibits a cellulose reaction similar to that found in *Parnassia* (**19**). The megagamete develops a big basal vacuole, and its nucleus comes to lie at its distal end (fig. 20).

The polar nuclei may remain together near the egg apparatus without undergoing actual fusion. Sometimes they can be found lying near the middle of the greatly elongated megagametophyte. They usually fuse before fertilization takes place.

A megagametophytic character conspicuous in *Lyonothamnus* is the chalazal elongation prior to fertilization. Among the Saxifragaceae such chalazal elongation is of rare occurrence. In some saxifragaceous genera (*Parnassia* **19,** *Philadelphus* **7**) there is more or less micropylar elongation of the gametophyte. As a rule the gametophyte of the Rosaceae shows a characteristic chalazal growth. In some of the rosaceous genera JACOBSSON-STIASNY (**13**) finds it to be either large and oval (*Spiraea, Sorbus, Rubus, Alchemilla, Rosa*), cylindrical (*Sanguisorba, Agrimonia*), or dumb-bell shaped (*Mespilus, Chaenomeles, Cydonia, Malus, Prunus, Cerasus*). While this chalazal enlargement found in *Lyonothamnus* is not universal among the Rosaceae, it is indicative of a rosaceous rather than of a saxifragaceous tendency.

<div align="center">SEED</div>

Attempts were made to trace the course of the pollen tube from the stigma to the ovule with the use of resorcin blue dissolved in 50 per cent alcohol. Paraffin sections of pistils collected at hourly intervals up to 48 hours after opening of the flowers did not yield results.

Such preparations showed pollen tubes for a few millimeters down the center of the style and in the nucellus, but they were not recognizable in the intervening tissue. As soon as the pollen tube reaches the nucellus, it grows to the base of and between the two synergids. No actual discharge of the microgametes nor fertilization has been observed. As soon as a zygote is formed its nucleus takes a basal position (fig. 22). The synergids show signs of degeneration with their filiform apparatus still distinct, their distal cytoplasm staining deep red, and their nuclei not at all recognizable.

While GRAY (8) reported at least one or two mature seeds in each follicle he examined, I do not find this to be the case. One or two apparently plump seeds may be found within a single follicle, but a microscopic examination shows that they contain no embryo. This is a common occurrence among Rosaceae, at least in the apple (17), pear (26), plum (10), cherry (27), etc., where the self-fertilized embryo fails to develop. No artificial pollination has been made in this investigation.

The zygote does not divide until some time after fertilization, when endosperm formation is well under way. Soon after fertilization the primary endosperm nucleus divides into two (fig. 23), one of which migrates to the chalazal end and the other remains at the middle of the embryo sac. As soon as the chalazal endosperm nucleus reaches the distal end of the embryo sac, there is a cytokenesis which cuts off a small cell at the lower end (fig. 24). Subsequent development is that usually found in a primary endosperm cell. There follow repeated divisions of the nuclei in the larger daughter cell (fig. 25), and a free cell formation follows this multinucleate stage. Without undergoing free nuclear divisions the small basal cell begins to divide, about the time of free cell formation in the upper cell, and eventually gives rise to a small conical mass of parenchymatous cells with dense homogeneous cytoplasm and distinct nuclei (fig. 26). This basal apparatus persists for some time, but eventually disintegrates (fig. 27) as the seed approaches maturity.

The similarity between the basal apparatus of *Lyonothamnus* and that of *Tillandsia usneoides* (1) is striking. Differentiation of the basal apparatus in *Lyonothamnus* takes place long after free cell formation is well under way, while the reverse is true for *Tillandsia;*

for example, the basal apparatus is formed soon after the first division of the endosperm nucleus.

Among the Saxifragaceae cell divisions may follow the first division of the primary endosperm nucleus, or its primary endosperm cell may become multinucleate before cell division. Soon after fertilization, the endosperm nucleus in *Heuchera purpurea* (7) divides at the chalaza; one part migrates to the center of the embryo sac while the other remains at the chalaza. This binucleate cell becomes unequally divided into two daughter cells, each of which divides repeatedly. In *Chrysosplenium alternifolium* (7) we also have the division of the primary endosperm cell into two unequal cells, in both of which free nuclear division takes place. Cell formation takes place first in the lower cell, from which a basal apparatus of about 50 cells (persisting as a crushed tissue in the mature seed) is developed. The same is true of *Saxifraga granulata* (7), except that an 8-celled basal apparatus is developed. In *Francoa appendiculata* (7) the whole embryo sac becomes filled with free endosperm nuclei; this is followed by cell formation only at the upper portion of the sac, while the basal end which functions as a haustorium remains multinucleate. From these fragmentary data it appears that the Saxifragaceae are mostly characterized by a division of the primary endosperm cell into two daughter cells, the lower end of which by one method or another develops into a basal apparatus.

Development of the endosperm among the Rosaceae begins with free nuclear division, which is later followed by cell formation. JACOBSSON-STIASNY (13) reports that the endosperm entirely fills the embryo sac, and this is later reduced by the developing embryo. In *Prunus* RUEHLE (21) finds an interesting endosperm formation. In this plant free nuclear divisions of the endosperm nucleus occur, and at the time divisions are about complete, cell formation takes place at the upper portion of the embryo sac, while the basal end remains nuclear and functions as a haustorium. The endosperm of *Lyonothamnus* is decidedly saxifragaceous in character, in that a cellular basal apparatus, a structure unknown in the Rosaceae, is formed.

Because of the scarcity of developing embryos, no detailed account can be given of the embryonal development. At the time the

cotyledons are becoming differentiated, the embryo has a massive suspensor 2–3 cells broad and 3–6 cells long. The basal cell of the suspensor is not enlarged. JACOBSSON-STIASNY (12) finds that in *Saxifraga* and *Chrysosplenium* a suspensor haustorium is formed. She cites GOEBEL as finding a suspensor haustorium in Ribesiaceae. GÄUMANN (7) pictures an embryo of *Chrysosplenium alternifolium* with two basal cells of the suspensor greatly enlarged. Among the Rosaceae, PÉCHOUTRE (20) finds no indication of an enlargement of the basal suspensor cell, except in *Spiraea filipendula*. In *Geum urbanum*, SOUÈGES (25) as well as PÉCHOUTRE (20) shows a suspensor of a row of cells 5–6 layers high. From the data at hand the embryo of *Lyonothamnus* seems to be more rosaceous than saxifragaceous in nature.

The Saxifragaceae and Rosaceae can be differentiated in the morphological structure of their mature seed. In Saxifragaceae GÄUMANN (7) observed that the inner layer of the inner integument is sometimes cutinized (*Francoa*) or else thickened (*Heuchera*), while the outer integument develops characteristic outgrowths which give the seed a jagged surface. Among the Rosaceae, PÉCHOUTRE (20) finds that there is never any sclerification or notable thickening of the inner integument, and that this tissue takes no particular part in the protection of the seed. The outer integument (except *Pirus* and "Amygdalées") has a sclerification in its outer layer. Some or few of the subepidermal parenchymatous cells persist unmodified; the other hypodermal cells are crushed and form a membranous layer.

The mature seed of *Lyonothamnus* (fig. 28) possesses a coat derived from both inner and outer integuments of the ovule. The innermost portion (fig. 29) derived from the inner integument is not thickened and contains tanniferous material. The remaining cells of the inner integument become crushed. The superficial layer of the outer integument becomes highly sclerified, the layer immediately beneath being composed of cells with unthickened walls and protoplasm rich in tannin. The remaining cells of the outer integument become crushed. The endosperm in the mature seed is composed of 7–9 layers of cells at its thickest portion and is thinner at both ends. Within the endosperm is an embryo which consists of a

large radicle directed against the micropyle, and a pair of fleshy planoconvex straight cotyledons. This feature of the mature seed of *Lyonothamnus* is decidedly rosaceous rather than saxifragaceous in character.

Summary

1. In its floral morphology *Lyonothamnus* possesses both saxifragaceous and rosaceous features. Development of the carpels before all the stamens are formed agrees with some of the Rosaceae and a few of the Saxifragaceae. The one-celled archesporium, the failure to form a "coiffe épidermique," the absence of an obturator, and the development of a cellular basal apparatus are features purely saxifragaceous in nature. The differentiation of many nucellar cells which exhibit characters typical of spore mother cells indicates a close relationship with the Rosaceae. The peculiar elongation of the megagametophyte prior to fertilization, and its seed characters (simple modification of the integument, large embryo with a pair of fleshy planoconvex straight cotyledons, unenlarged basal suspensor cell, and sparingly developed endosperm) are features found among the Rosaceae.

2. While many of the floral characters of *Lyonothamnus* are either saxifragaceous or rosaceous in nature, the formation of two series of ovules in its ovary is a morphological criterion by which ENGLER and PRANTL (5) separate the Cunoniaceae from the Saxifragaceae. The family characteristics of Cunoniaceae and Saxifragaceae are so closely alike that early taxonomists considered Cunoniaceae a tribe of the Saxifragaceae. It seems evident that we are dealing here with a genus possessing heterogeneous morphological characters which tend to obliterate the distinction between Rosaceae and Saxifragaceae, and at the same time possessing a cunoniaceous arrangement of ovules.

3. It is unfortunate that no morphological studies of genera closely related to *Lyonothamnus* in both Rosaceae (*Vauquelinia, Lindleya, Photinia*) and Saxifragaceae (*Jamesia, Fendlera*), as well as those in the Cunoniaceae, have been made. Until such studies are made, *Lyonothamnus* should perhaps stand as a transitional form between Saxifragaceae and Rosaceae on the one hand, and Cunoniaceae on the other.

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THE LAYERING HABIT IN SITKA SPRUCE AND THE TWO WESTERN HEMLOCKS

WILLIAM S. COOPER

(WITH FOUR FIGURES)

In a paper published in 1911 (1), I discussed the habit of layering among conifers, especially as observed upon Isle Royale, Lake Superior. The scanty literature of the subject was also summarized, thirteen references, mostly European, being found. From the literature and my own work the following conclusions were reached. The habit of layering is common among coniferous trees, and particularly characteristic of the genera *Picea* and *Abies*. It occurs more frequently and attains more striking development with increasing latitude and altitude. Because of its effect upon the abundance and character of the reproduction, it is often an important factor in forest dynamics.

Since 1911 three further contributions have appeared. In 1913 FULLER (3) published a brief account of layering in the black spruce (*Picea mariana* (Mill.) BSP) as observed in the Saguenay region of Quebec. According to this report, the habit is characteristic of trees growing in open stand upon bare granite hills, each trunk being surrounded by a thick mat of basal branches, many of which root and become erect, so that the parent acquires a ring of daughter trees. Sometimes the original trunk dies and the result is a circular area covered with vigorous young upright shoots. The habit is of considerable importance in the establishment of forest upon bare granite areas.

In 1923, in a paper (2) dealing with forest development after glacier retreat at Glacier Bay, Alaska, I included a brief description of layering in the Sitka spruce (*P. sitchensis* Carr.). LUTZ, in 1930 (5), noted an instance of layering in the mountain hemlock (*Tsuga mertensiana* Carr.).

During a more recent visit to Glacier Bay, in the summer of 1929, many new facts came to light, so that a further report seems justified. It was discovered that the coast hemlock (*Tsuga heterophylla*

Sarg.) and *T. mertensiana* also layer, although the Sitka spruce does so most abundantly and vigorously.

The spruce, when growing in a pioneer environment, first develops a low bushy form, with the oldest branches lying on the ground. It is only such open-grown individuals that layer, since healthy basal branches are necessary. Rooting, however, will not occur until the prostrate branch becomes well covered by plant waste, and this must require several growing seasons at least. The branch may thus attain a considerable horizontal length before the change in geotropic response occurs. Adventitious roots are finally produced, the branch turns erect, radial symmetry is substituted for dorsiventral, and a treelike shoot is the result.

In areas transitional between willow thicket and forest, one finds apparent groups of young spruces which in reality are made up of a parent tree surrounded by layered shoots of almost equal height. Such a group is illustrated in my earlier paper (2, fig. 8). The branches which produce the daughter trees leave the main trunk at the surface of the ground or a little above it, or are completely buried by vegetable débris. The horizontal branch is slender close to the trunk, but becomes progressively thicker outward, reaching a maximum in the neighborhood of the upward bend (fig. 1). Under such open conditions, where there is an approximation to equality between parent and daughter shoots, any or all may form a part of the mature stand when the community becomes a closed forest. Where the parent trunks are more closely placed, the layering shoots, although originating as basal branches of open-grown trees, soon become overshadowed by the development of their parents, and persist for many years beneath the dense canopy in suppressed condition. Even these have their possibilities, however, for the destruction of one or more of the parent trees may provide an opportunity for vigorous renewal of growth.

Such an area was found on the east shore of Glacier Bay, just within the entrance (station 52). The appearance here was of a closed stand of submature spruce with frequent seedling trees of the same species beneath. It was soon discovered, however, that every apparent young tree was in reality a layered branch. A quadrat 10 m. square was laid out and a complete survey made (fig. 2). The

area was controlled by four spruces, ranging in diameter from 42 to
58 cm. and in age from 51 to 82 years. A single coast hemlock was
present, 29 cm. in diameter and 58 years old. Three relict clumps of
willow and one of alder, all low in vitality, still survived. The char-

FIG. 1.—Layered branch of *Picea sitchensis*, Strawberry Island, Glacier Bay, Alaska

acteristic ground cover of forest mosses was rather well developed,
and the herbs, as usual, were few. The layering branches were found
to leave the trunk below the present soil surface, and the horizontal
portions, lying approximately on the surface of the mineral soil,
were entirely concealed beneath several inches of litter. All the erect
shoots showed the effects of suppression, but in differing degree. The
average width of ring for the five dominant trees was 2.92 cm.; of
the thirteen layering shoots, sectioned at the base of the erect por-

tion, 0.437 cm., with an extreme range of 0.205–1.141 cm. Suppression must be due, not to any direct dependence on the parent trunk, but to competition between parent and daughter shoots behaving as independent individuals.
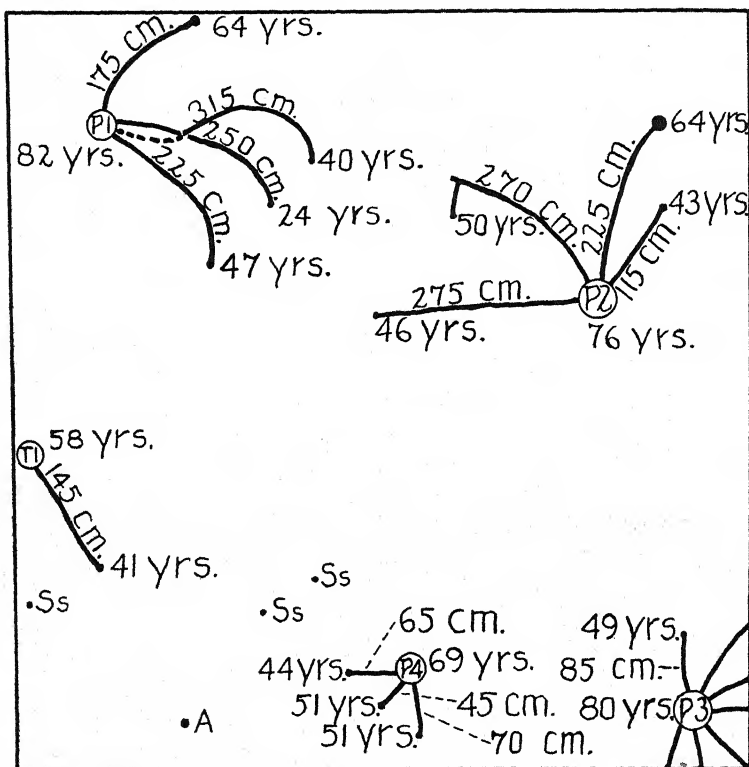
FIG. 2.—Quadrat in submature forest, east shore of Glacier Bay, Alaska, 10 m. square; parent trees and layering branches: *P = Picea sitchensis; Th = Tsuga heterophylla; A = Alnus tenuifolia; Ss = Salix sitchensis.*

Sections of the layering shoots show consistently a brief initial period of slow growth followed by a period of comparatively rapid increase, probably associated with the development of abundant adventitious roots before the forest canopy had become thoroughly closed. The final rings, belonging to the period of suppression, are almost microscopic. The length of the horizontal portion varied

from 45 to 315 cm. One erect shoot had developed from a secondary branch, the original terminal having been destroyed. Difference in age between parent and daughter shoot ranged from 8 to 58 years. Most of this is accounted for in the period of horizontal growth, but a portion is due to unavoidable uncertainty in counting because of closeness and incompleteness of rings. Undoubtedly these shoots have at least as good a chance of surviving and becoming members of the dominant stand as would seedlings under like conditions, and the layering habit is therefore of some consequence in the dynamics of the forest, here as on Isle Royale. The extreme likeness in appearance to seedling reproduction constitutes a warning against the drawing of too hasty conclusions in such a forest.

Two small layering branches, one from a spruce, the other from a coast hemlock, were brought from Glacier Bay and subjected to detailed analysis. The first was collected at station 22, in an area transitional between willow thicket and spruce forest, dense shade having not yet developed. The horizontal portion was 80 cm. long and the erect shoot 80 cm. high. Adventitious roots were present at intervals along the horizontal portion, mostly but not exclusively grouped in the vicinity of the annual bud scars. The diameter of the branch at the surface of the parent trunk was 7 mm., the cross-section being circular and continuing so as far as the third bud scar. At the second bud scar began a gradual increase in diameter, which continued to a point just short of the upward bend. Sudden increases in thickness were evident where the principal roots originated, and, in addition, local swelling also occurred at these points. Asymmetry began to show itself at the third bud scar, the vertical diameter increasing faster than the horizontal, this condition persisting through the region of the bend and disappearing in the erect portion. Maximum asymmetry occurred between the fourth scar and the bend, the average dimensions of three cross-sections being 13 × 17 mm.

This branch was sectioned for microscopic study at the surface of the trunk and just beyond each annual bud scar. The results are presented diagrammatically in fig. 3 A, no attempt being made to indicate the varying thickness of the rings. It was possible to trace groups of rings and in many cases single ones from section to section, and thus to work out with considerable exactness the history of the

branch. Triangle *a-d-e* represents a central cone of wood (or rather one-half of it) made up of five wide rings in section o, followed by four, three, two, and one in the succeeding sections. It is evident that in the first five years of its life (plus a probably brief period represented by the portion buried in the trunk) the branch grew rapidly, obtaining a length of 65 cm. During the next eight years (*d-e-c-b*) growth was slower, 43 cm. of length being added and a thickness of wood less than the total product of the previous five
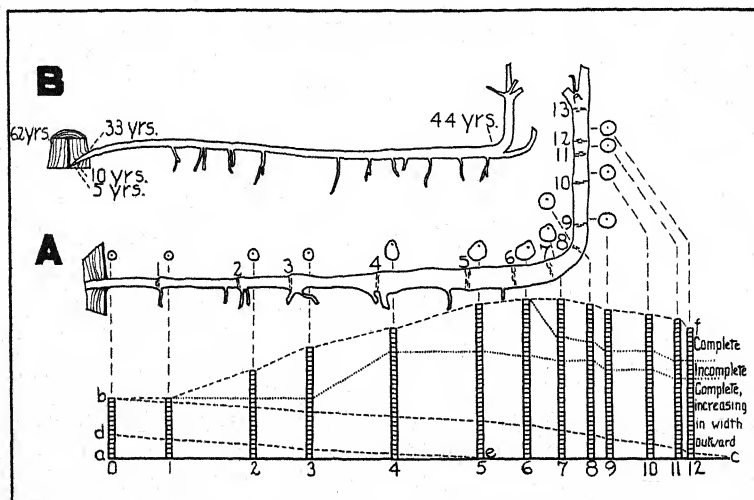
FIG. 3.—*A*, anatomy of layered branch of *Picea sitchensis*, Glacier Bay, Alaska; *B*, layered branch of *Tsuga mertensiana*, Glacier Bay.

years. After the thirteenth year growth near the trunk ceased, but new rings were added in the outer portion.

The triangle *b-c-f* indicates the wood increment after growth near the trunk had ceased. We have here the anomaly of an increase in the number of growth rings with increasing distance from the parent trunk. The evident cause is the acquisition of an independent absorbing apparatus in the form of adventitious roots. As to the location of the first-formed of these, with resulting first additional wood production, it is impossible to be certain. The largest roots were those near the third and fourth bud scars, and the most abrupt enlargement is associated with these. The sudden increase in the

new set of complete growth rings between sections 3 and 4 is a significant point. The outer half of the branch, too, because of its weight, would be likely to be first covered by litter. The lesser amounts of wood nearer to the trunk might well have been added later, as this portion finally became buried and produced roots.

The branch tip seems to have turned erect at about the eighth year. It is true, of course, that an independent change of growth direction does sometimes take place in a matured wood structure, but in the present case the evidence indicates that the change was accomplished by the growing tip itself within the space of a year or two. The initiation of eccentricity, and so doubtless the first production of adventitious roots, dates from the same year; and beyond the ninth scar there is no perceptible eccentricity, even in the earliest rings.

It is possible to date any ring in any section by adding the number of the ring in the section and the number of bud scars between it and the trunk, assuming the innermost ring of section 0 as number one, and assuming also, of course, continuity of growth. Using this method we find that pronounced eccentricity first became manifest between the eighth and thirteenth years in the life of the branch. Two periods are sharply distinguished in the time during which the adventitious root system functioned. The first was characterized by the production of complete but strongly asymmetric rings. During this period the annual wood increment gained in thickness from year to year almost without interruption. In the horizontal portion by far the greatest amount was added to the lower side; in the erect portion (sections 9–12) there was an equivalent increase in wood production, but with equal development along all radii.

In about the twenty-eighth year the period of rapid asymmetrical growth came to an end. It was followed by a time of exceedingly meager wood production, with complete failure on the upper side, resulting in a group of partial and very narrow rings. The suddenness of the change is emphasized by the fact that the first ring of this period appears in the sections as an arc of less than 150°. The second overlaps the first to some extent, and the succeeding rings in many cases closely approach completeness. In the horizontal portion the rings remain incomplete to the last, increasing somewhat in width,

then decreasing, at the same time becoming narrower in circumferential extent (fig. 4). In the upright portion, four incomplete rings are succeeded by a second set of wider complete ones. The sudden decrease in wood production must be attributed to the onset of unfavorable conditions in environment or in the plant itself. There is
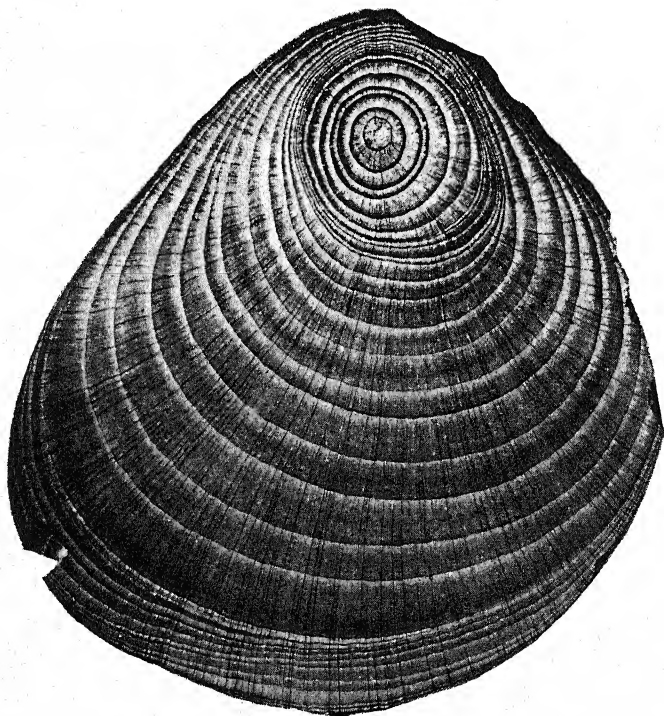
FIG. 4.—Section of layered branch of *Picea sitchensis* (section 5 of *A*, fig. 3)

no clue as to its nature. Partial recovery brought about in the horizontal portion a radial widening of the incomplete rings; in the upright portion both widening and completion.

A small layering branch of mountain hemlock was also studied (fig. 3*B*), and in this it was possible to trace the branch to its origin at the center of the trunk. At two points within the trunk the branch showed five and ten rings; at the surface of the trunk 33, and just before the bend 44. The horizontal portion was about 75 cm. long and bore scattered roots from a point 30 cm. from the trunk as far

as the bend. The erect portion was 45 cm. tall. This branch showed the same features as the last: a circular cross-section near the trunk and an elliptical section near the bend, with most of the new wood added to the lower side. A very uncertain count of bud scars indicates a minimum age of 11 years to the point, just short of the bend, where 44 rings occur. A minimum age of 55 years is thus established for the age of the branch as a whole. The age of the trunk at the point of origin of the branch is 63 years. Eight years are thus unaccounted for, which may be due either to the impossibility of an accurate count or to the complete failure of wood formation at some time during the history.

It is worth while to attempt a physiological explanation of the phenomena displayed by these layering branches. Conditions incident to burial in vegetable débris cause the production of adventitious roots, principally upon the lower side. The use of foods in root building causes a general flow of these substances toward the roots, and a retarding or complete stoppage of flow back into the trunk. Practical cessation of growth in the proximal portion of the branch results. The adventitious roots pour water into the lower side of the horizontal branch. The water takes the line of least resistance and flows mainly through the lower part of the branch toward the growing tip. As a response to vigorous water flow and ample food supply, the production of wood cells should be most abundant on the lower side of the branch beyond the point of origin of the innermost important adventitious roots. The distal portion of the branch thus becomes eccentric, with the main body of wood below the growth center. It is true that in many cases gravity alone is sufficient to produce eccentricity in a horizontal organ. Experimental work now in progress shows this to be the case in the branches of Monterey cypress (*Cupressus macrocarpa*). In these spruces and hemlocks, however, the perfect circularity of the proximal portion of the branch shows that gravity alone is not the cause; the development of eccentricity is plainly connected with the production of adventitious roots.

As to the cause of the upward bend, an explanation suggests itself which is extremely plausible so far as the present cases are concerned: that the bending is due to faster growth on the lower side in the region of the tip where the tissues are still plastic. The point at

which it occurs is determined by the time when the flow from the newly formed adventitious roots first makes itself felt. When perpendicularity is attained the flow is no longer differential, the new position is made permanent by the fixation of tissues, and eccentricity gives way to perfect concentricity. Radial symmetry in the arrangement of the secondary branches is a final result. This explanation has the merit of extreme simplicity, and one is tempted to apply it to all the numerous cases in which lateral shoots, ordinarily plagiotropic, suddenly become orthotropic. GOEBEL (4) offers a somewhat similar but less definite theory, which is discussed in my former paper (1). GOEBEL thinks that a vigorous stream of water and other nutritive materials induce the orthotropic habit, and that the main shoot of a plant is therefore erect, while the less abundantly nourished laterals are plagiotropic. When the terminal shoot is removed the main stream is deflected into the uppermost lateral, which in consequence becomes erect. In the case of a layering branch the increased flow is due to the activity of the adventitious roots, the result being the same. GOEBEL's explanation is rather vague in its mere assumption of a general stimulus; the suggestion I have offered at least provides a possible mechanism.

Unfortunately for both, there are a few cases which seem to range themselves in opposition. One, described in my former paper (1), was a balsam fir that grew in an open rock crevice. Several of the lower branches descended into the crevice a short distance, finally turning erect and developing a treelike form. No soil was present, no roots were produced, and the branch remained entirely dependent upon the parent trunk. In dense forest at Glacier Bay I found numerous seedling plants of mountain hemlock which took the form of prostrate mats, rooting abundantly but showing no tendency to turn erect.

The problem is of considerable physiological interest, and is evidently not a simple one. It would seem to be susceptible of experimental investigation, and Sitka spruce is recommended as promising material for such a study.

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ORIGIN AND DEVELOPMENT OF TISSUES IN STEM OF SELAGINELLA WILDENOVII

Bertram Donald Barclay

(WITH NINETEEN FIGURES)

Introduction

Much work has been done on the anatomy of the mature structures in *Selaginella*, but the literature of the subject includes little information as to the exact origin of these tissues. The recent work of CHANG (2) on *Pteris aquilina*, and JOHNSON (4) on *Equisetum*, show many points at variance with the older accounts, especially as to what constitutes the exact limit between stele and cortex, and as to the origin of the endodermis and pericycle.

Selaginella was chosen as a subject of investigation for various reasons. It represents a group of plants generally possessing a single apical cell, which facilitates tracing the tissues to their exact origin. It belongs to a division of pteridophytes that has not been critically investigated for many years, while members of the Filicales and the Equisetales have only recently been reworked. Also the existing accounts of tissue origin and development in *Selaginella* do not agree, indicating the need of further work. Of the available species, *Selaginella wildenovii* Bak. was found to be the most favorable for this study. The young primary branches grow to a greater length without forking than in other species available. The individual cells are also larger than in other species.

This investigation deals first with the general gross and microscopic anatomy of the young primary stem, then with the apical cell and its derivatives, the origin and development of cortex, endodermis, and air spaces, pericycle, xylem, and phloem. The origin and development of the xylem and phloem are discussed only briefly at this time.

Material and methods

Collections were made at the University greenhouses during the winter months, since at this time of year *Selaginella wildenovii*

makes its most vigorous vegetative growth. The best fixation was obtained by the use of a formalin-acetic-alcohol mixture. The saf-ranin-light green combination was found to be the most successful anatomical stain.

Investigation

General anatomy

The stem of *Selaginella wildenovii* is flattened dorsiventrally. Two rows of small leaves are borne on the dorsal surface, and two rows of larger leaves on the ventral surface. A view of the dorsal surface of a young primary shoot shows that the young growing tip always bends upward, the whole stem curving slightly in this direction. The position of the growing point and the upward curvature of the stem make impossible a median longitudinal section parallel to the dorsiventral plane, and make difficult a cross-section through the apical cell. The median longitudinal section figured is cut at right angles to the dorsiventral surface.

In a well developed primary branch, the vascular cylinder consists of a single protostele, flattened dorsiventrally, with two lateral protoxylem points (fig. 3). The metaxylem develops centripetally and meets in the center of the stele. The xylem is entirely surrounded by one or two layers of protophloem, easily recognized by the unusually bright green color of the walls. The central cylinder is surrounded by the pericycle, generally one layer of cells in thickness. The cells of the pericycle contain a single chloroplast. Between the pericycle and the inner cortex is a broad air space, bridged by the elongated endodermal cells or by an endodermal cell united with one or more elongated cells without endodermal thickenings. These cells have a common origin with the endodermal cell, but unlike it do not develop Casparian thickenings, and may possess chloroplasts. The cells composing the inner layer of the cortex contain a single large chloroplast, U-shaped in cross and longitudinal section, and next to the cell wall that abuts the air space. All the other cortical cells contain chloroplasts, but these are smaller and different in form from those in the cells of the inner cortex.

APICAL CELL AND ITS DERIVATIVES.—The stem grows by means of a tetrahedral apical cell. A transverse section of this apical cell
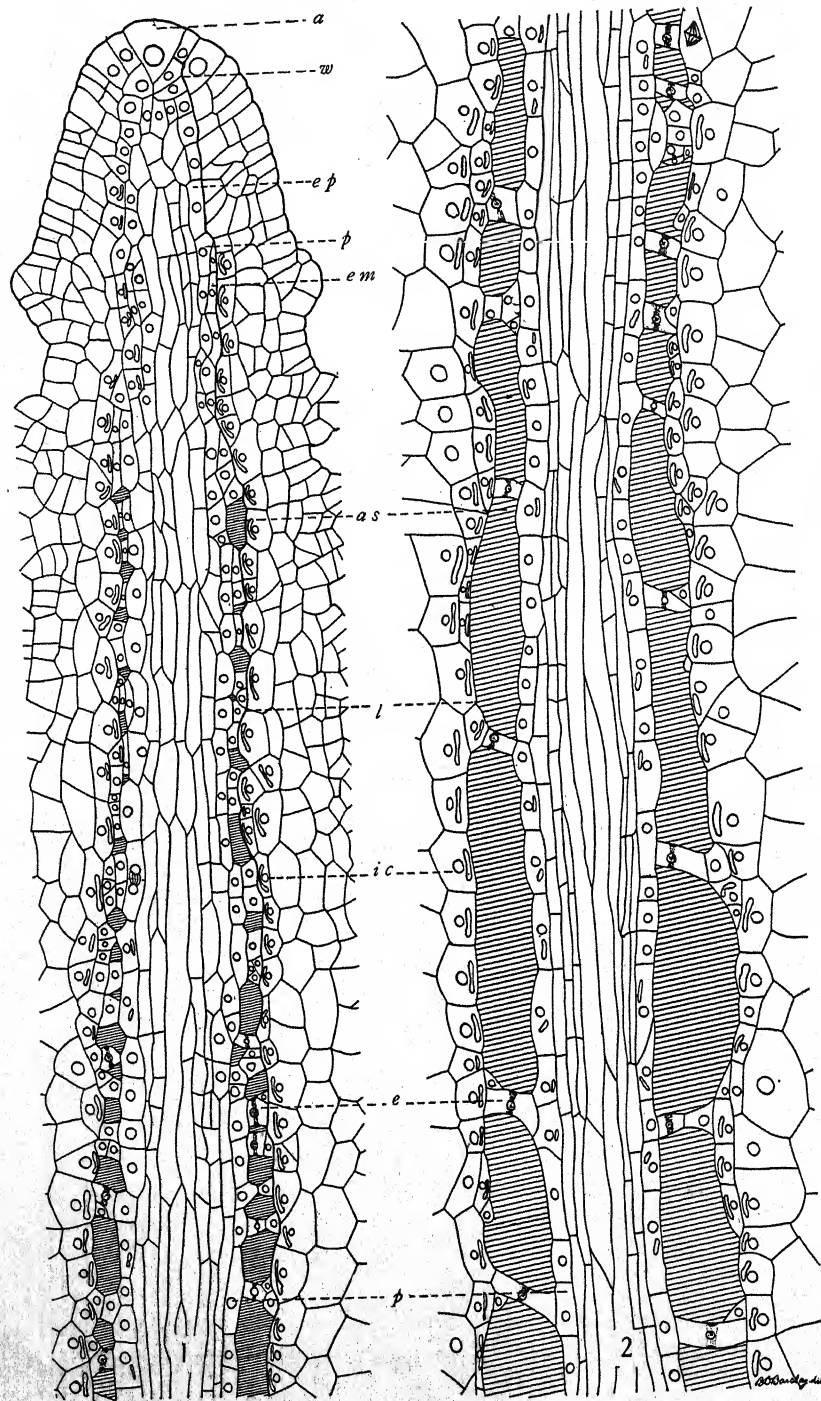
FIGS. 1, 2.—Median longitudinal section of young shoot at right angles to dorsiventral surface: *a*, apical cell; *w*, periclinal wall dividing first segment into stelar and cortical segments; *ep*, cell giving rise to endodermis and pericycle; *p*, pericycle; *em*, endodermis mother cell; *e*, endodermal cell; *as*, air space; *ic*, inner cortex; *l*, limit between stele and cortex; ×420.
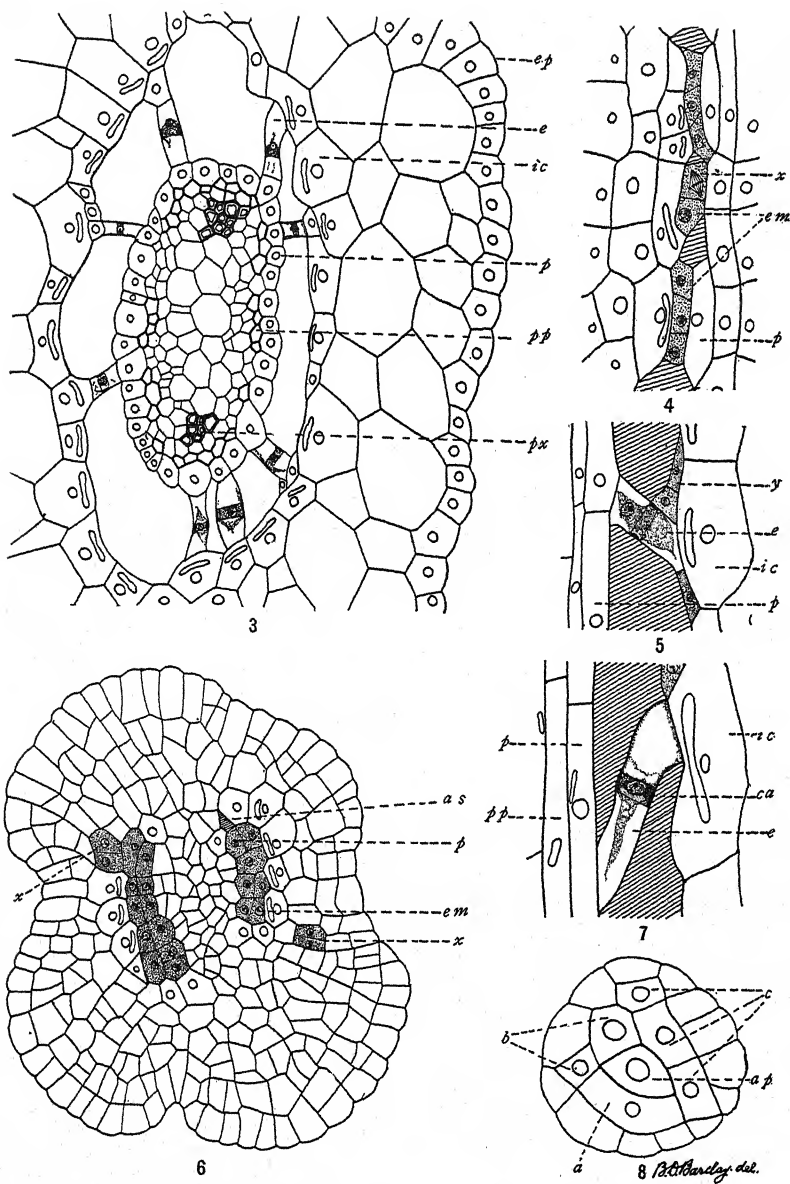
FIGS. 3–8.—Fig. 3, transverse section of young shoot, showing epidermis (*ep*), inner cortex (*ic*), endodermal cells (*e*), pericycle (*p*), protophloem (*pp*), protoxylem (*px*); ×570. Fig. 4, longitudinal section showing inner cortex to left of endodermis mother cells; endodermal mother cell (*x*) dividing by oblique wall (fig. 13) shows stage immediately following oblique division); ×655. Fig. 5, longitudinal section showing endodermal cell (*e*); stippled cells (*y*) are derivatives of endodermal mother cells; shaded areas indicate air spaces; ×570. Fig. 6, transverse section through young shoot at level at which endodermis and pericycle are first differentiated; ×570. Fig. 7, simple endodermal cell (*e*) stretched obliquely by differential longitudinal growth of central and cortical cylinder; *ca*, Casparian strip; ×655. Fig. 8, transverse section of apical cell and segments; *a*, first segment; *b*, second segment; *c*, third segment; cells composing second segment distorted by growth; ×655.

is shown in fig. 8. Seen in longitudinal section (fig. 1), the first segment of the apical cell divides by means of anticlinal and periclinal walls. The desmogen strand arises from the products of the inner half of the periclinal division, while the products of the outer half remain parenchymatous. The desmogen strand can be distinguished from the rest of the tissue five or six divisions below the apical cell (fig. 1).

CORTEX.—The outer cell resulting from the first division of the first segment of the apical cell forms by tangential and transverse divisions a cortical tissue four or five cells in thickness. The cortical cylinder increases in size by radial divisions of its cells (fig. 6), but the number of layers of cells does not increase for a long time. This increase of internal diameter of the cortical cylinder, together with its growth in length, brings about the formation of air cavities and the separation and stretching of the endodermal cells, as will be shown later.

ENDODERMIS AND AIR SPACES.—The endodermal cells can first clearly be recognized about 0.3 mm. from the stem tip by the Casparian strips which line the radial and end walls of the endodermal cell (fig. 1 e). In sections stained with safranin and light green, the Casparian strips are seen first as reddish points on the radial and cross walls, to which the cytoplasm adheres when the cells are plasmolyzed (fig. 19). The nucleus of the endodermal cell at this stage always lies in the plane of the thickenings (figs. 10, 19). An endodermal cell can be recognized several cells above the place where the Casparian thickenings first appear, by the manner in which plasmolysis occurs (fig. 15). The endodermal cell may be united directly to the pericycle on one side and to the inner cortex on the other (figs. 7, 11, 14). It may also be united directly to the pericycle on the inner side, and joined to the inner cortex by means of one or more cells which arose from a single endodermis mother cell.

The cells of the inner cortex with their conspicuous chloroplasts aid in tracing the inner limit of the cortex (fig. 1 ic). The inner layer of the cortex can thus be traced to within five cell divisions of the apical cell. From this point on it is an easy matter to trace the cell which will give rise to the endodermis (endodermis mother cell), to its common origin with the cell which will give rise to the peri-
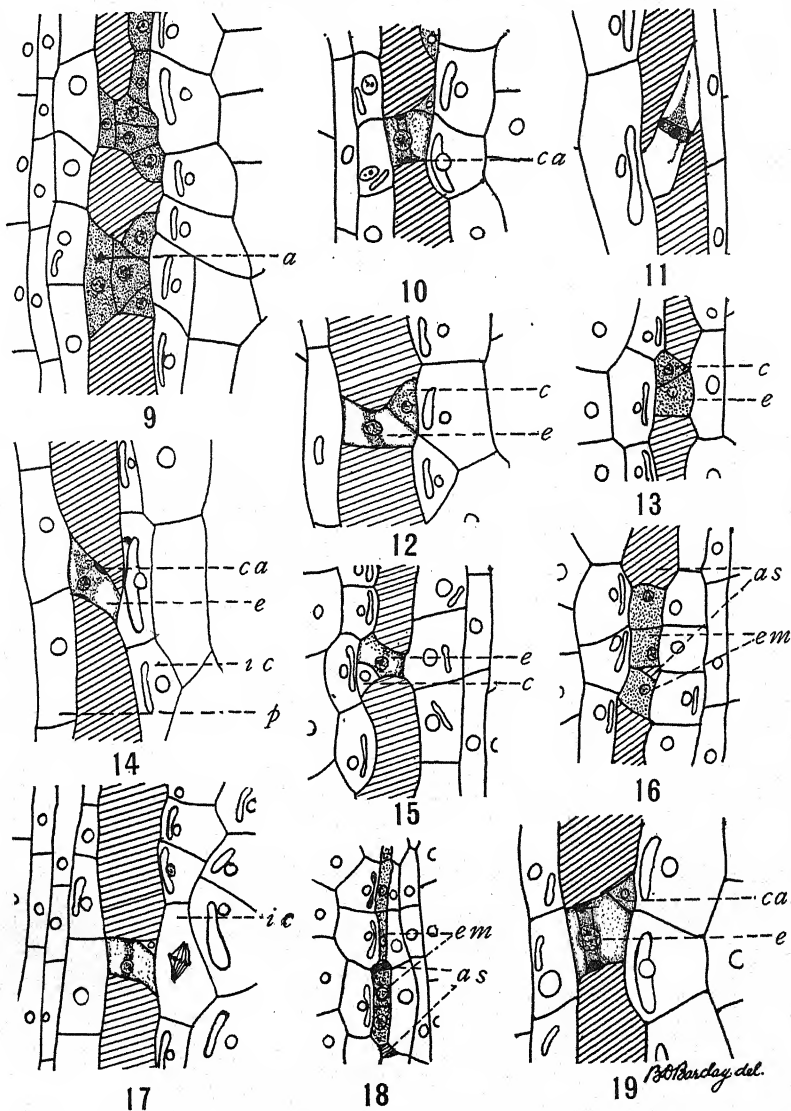
FIGS. 9–19.—Fig. 9, longitudinal section showing derivatives of endodermal mother cells. Cell (*a*) will become endodermal (this cell lies next to pericycle); ×810. Fig. 10, endodermal cell shortly after appearance of Casparian strip; ×810. Fig. 11, obliquely stretched endodermal cell similar to that in fig. 7; ×550. Fig. 12, longitudinal section showing result of oblique division of endodermis mother cell shown in fig. 4; *e*, endodermal cell; *c*, sister cell. Fig. 13, similar to fig. 12, but showing earlier stage following oblique division of endodermis mother cell. Fig. 14, endodermal cell similar to that in fig. 7. Fig. 15, cells formed by oblique division of endodermis mother cell, similar to fig. 12. Fig. 16, longitudinal section showing formation of air spaces between endodermis mother cells. Fig. 17, longitudinal section showing elongation of inner cortex by transverse division. Fig. 18, longitudinal section showing formation of air spaces between endodermis mother cells; pericycle to right of endodermis mother cells, inner cortex to left. Fig. 19, longitudinal section through endodermal cell, showing first appearance of Casparian strip; figs. 12–19, ×810.

cycle. The mother cell of the endodermis and pericycle can be traced directly to the inner cell resulting from the periclinal division of the first segment of the apical cell. This inner segment gives rise to the initial cell of the endodermis and pericycle and the initial cell of the xylem and phloem.

The cells which later give rise to endodermis, at first form a compact tissue. Soon radial divisions occur in the cells of the cortex (fig. 6), and the whole cortical cylinder increases in diameter. Longitudinal growth of the cortical cylinder occurs at the same time. The desmogen strand, together with the pericycle, increases in diameter, but more slowly than the cortical cylinder. The increase in length of the part of the stele inclosed within the pericycle keeps approximate pace with the elongation of the cortical cylinder. The rate of growth of the endodermis mother cells does not keep up with the differential increase in diameter and length of the cortical cylinder and central bundle. As a result, the endodermis mother cells become stretched radially between the pericycle and inner cortex, and their radial walls become separated in the vertical and horizontal planes (figs. 1, 4, 6, 16, 18). An air cavity is thus formed between the pericycle and inner cortex, bridged by the endodermis mother cells. The endodermis mother cell may give rise directly to a single trabecula with its endodermal thickenings (figs. 7, 11, 14), uniting the pericycle to the inner cortex. The endodermis mother cell may also divide longitudinally and give rise to a trabecula composed of a single cell with endodermal thickenings next to the pericycle, and one or more cells uniting it to the inner cortex (fig. 9). An oblique wall may come in at the first division of the endodermis mother cell (fig. 4). In this case the daughter cell that has the firmest attachment to both pericycle and inner cortex stretches and becomes endodermal, while the other daughter cell is pulled away from the pericycle and lies next to the cortex (figs. 5, 10, 12, 13, 15). Occasionally a group of endodermis mother cells may become separated from the pericycle at an early stage and remain as a thin layer of cells against the inner cortex (fig. 9). This is the "trabecular cortex" of VLADESCU (9). Chloroplasts were not observed in cells with endodermal thickenings, but were found in other trabecular cells.

PERICYCLE.—The cells of the pericycle are at first of the same dimensions as the cells of the neighboring desmogen strands and the endodermis mother cells. The desmogen cells divide vertically and become much narrower than the pericyclic cells (fig. 1). The endodermis mother cells do not increase in length, and become stretched and separated. By this time the pericycle forms an easily distinguishable tissue. The pericyclic cells contain a single flattened chloroplast, generally located against the cell wall away from the air space.

XYLEM AND PHLOEM.—The first protoxylem to appear can be recognized in the leaf traces. The first protoxylem elements in the stem make their appearance a short distance below the first lignification in the leaf trace. This occurs about 0.3 mm. below the stem tip. The first protophloem appears about 0.05 mm. below the first protoxylem. Lateral sieve plates were observed in the protophloem sieve tubes. Metaxylem and metaphloem are differentiated much later.

Discussion

Various types of apical cells have been reported in the Selaginellaceae. PFEFFER (5) found that the stem of Selaginella martensii grows by means of a 2-sided apical cell. TREUB (7), working on the same species, reported that growth took place by means of either a 2-sided, 3-sided or 4-sided apical cell. HOFMEISTER (3) found a 2-sided apical cell in S. hortensis and S. galeotii. The writer finds that S. wildenovii possesses a 3-sided apical cell. BRUCHMANN (1), in his studies on S. spinulosa, found apical growth taking place by means of a general meristem, as in the Lycopodiaceae. STRASBURGER (6) reported that S. wallichii possesses a group of two initial cells. This seems to show that there is in Selaginella every intergradation, from a single apical cell to a general meristematic group. The different kinds of apical cell reported, and the variance of opinion on the type of apical cell possessed by one species, indicate that the apical situation in the genus as a whole is in need of careful reinvestigation.

VAN TIEGHEM (8) found that in Selaginella inaequifolia, S. wallichii, and others, each segment of the apical cell divides by means

of a tangential wall, then by a second periclinal division exterior to the first. Of the three segments thus formed, the inner one gives rise to the central cylinder and pericycle. The middle segment forms the endodermis and inner cortex, and the outer segment produces the outer cortex and epidermis. He reasons from this that the endodermis forms the innermost layer of the cortex and the pericycle the outermost layer of the stele. He adds that this situation is general for the ferns and specific for *Marsilea*, *Selaginella*, and *Equisetum*. Although he gives an authoritative account, no figures showing his findings in the stem are presented.

VLADESCU reports a different situation in *Selaginella*. His report describes in detail the origin of tissues, but he gives no figures and does not mention the species with which he worked. According to his findings, the first segment of the apical cell (form not given) divides into three segments, as reported by VAN TIEGHEM. The outermost segment forms the epidermis and the outer cortex. The middle segment forms the pericycle, endodermis and its derivatives, and the inner cortex, while the innermost segment gives rise to the vascular tissues. He finds a common origin for endodermis, pericycle, and inner cortex, and reports that the endodermis and pericycle arise from a single mother cell. His conclusion is that the line between stele and cortex does not divide the endodermis from the pericycle, but that the stele includes the endodermis and inner cortex. STRASBURGER agrees with VLADESCU's findings, and adds that he had previously arrived at the same conclusions in his work on *Selaginella* and *Lycopodium*.

In *Selaginella wildenovii*, which possesses a tetrahedral apical cell, the first segments of the apical cell divide tangentially to form only two segments. The outermost gives rise to cortex only, and the innermost forms the vascular elements, pericycle, and endodermis with its derivatives. The limit of stele and cortex in this species is thus between the endodermis and the inner cortex. This situation corresponds exactly with the findings of CHANG (2) in *Pteris aquilina*.

In view of these conflicting reports, it is evident that much work on the origin and development of tissues in this genus is still needed.

Summary

1. The stem of *Selaginella wildenovii* grows by means of a tetrahedral apical cell.

2. The endodermis, pericycle, and vascular strand have a common origin and are all stelar. The endodermis forms the outermost layer of the stele.

3. The endodermis and pericycle arise from a common initial.

4. Elongation of the endodermal cells and formation of air spaces are explained by the mechanics of differential cell growth.

5. The protoxylem differentiates first in the leaf traces and later in the stem. The protophloem differentiates slightly later than the protoxylem.

The writer wishes to express his indebtedness and gratitude to Professor W. J. G. LAND, at whose suggestion and under whose direction this study has been made.

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BRIEFER ARTICLES

A HYBRID LOBELIA

Reference is made in the manuals to hybrids of *Lobelia syphilitica* L. and *L. cardinalis* L., but not to hybrids of the first species with *L. puberula* Michx. Plants of *L. syphilitica* and *L. cardinalis* were grown for many years in the wild flower garden, with no instance of crossing. The more recent introduction of *L. puberula* into the garden has resulted in the appearance of a number of forms of blue lobelia which can be explained only as hybrids. These hybrid plants (*Lobelia syphilitica* L. ✕ *puberula* Michx.) are densely and finely puberulent throughout, and the larger anthers are minutely bearded, characters of the *puberula* parent. In stoutness and leafiness of stem, the plants resemble *L. syphilitica*. The flowers are nearly the size of those of *L. syphilitica*, with the stout tube and strongly corrugated lower lip. The calyx resembles that of *L. syphilitica* much more than it does *L. puberula*, the size of the strongly deflexed auricles varying somewhat on different plants. Leaf shape is intermediate between the leaf shapes of the two parents, the apex being more like that of *puberula*.

While this hybrid form has not been observed in the field, it may be expected where the two species occur together. From a distance it might be mistaken for a plant of *L. syphilitica*, but on feeling the leaves, the touch would at once suggest *L. puberula*.

Among the segregates of the *Lobelia syphilitica*✕*puberula* hybrid a considerable range of forms is evident. Plants vary in respect to some characters, as leaf thickness and obtuseness of apex, form of calyx tube, size and shape of auricles, flower color, and form of lower lip. This last, however, in every case shows the divergent sides evident in fresh specimens of *L. puberula*, in contrast to the parallel or even slightly convergent sides of the lower lip of *L. syphilitica*. Characters of the *puberula* parent which appear to be dominant, prevailing in all forms although varying slightly in intensity, are puberulence of leaves and stem, puberulence of anthers, and one-sided form of inflorescence. The arching stamen tube of *L. syphilitica* does not appear in any of the forms observed.

While most of the segregates appear to be somewhat intermediate, although more strongly resembling *L. puberula*, one departs markedly in

flower aspect from the others. The flowers are larger than in either parent (about 3 cm. long); the lobes of the lower lip are broad and widely spreading; the inflorescence is compact (as in *syphilitica*) but tending to secund (as in *puberula*); the anthers are puberulent only on the angles; the auricles of the calyx are large and the calyx tube hemispherical as in *L. syphilitica*. This form surpasses in beauty either of the parents.

As the observations here recorded are based on only chance occurrence of plants, it is of course possible that some more recent forms are the result of second crossing and are not true F_2 segregates.

Such a variety of forms is at least possible, however, and it may be of interest to taxonomists that the combination of characters in at least one of them is such that it could not be ascribed to any species by the use of keys and manuals.—E. LUCY BRAUN, *University of Cincinnati, Cincinnati, Ohio.*

CURRENT LITERATURE

BOOK REVIEWS

Chemical Plant Physiology

Botanists, and especially plant chemists, will welcome Lyon's translation[1] of Kostychew's *Chemische Physiologie*. The emphasis on chemical plant physiology is one of the recent important developments in plant physiology. As brought out by the author, this has been made possible by late contributions in physical and biological chemistry. The result is that modern textbooks of plant physiology present a different aspect from those published a few decades ago. Much more attention is given to the chemistry of plant processes and less to various phases of growth and movement, the physics and chemistry of which are very poorly understood. As stated by the translator, the German volume was published in 1926 as Part I of the author's *Lehrbuch der Pflanzenphysiologie*, the implication being that there would be a second volume dealing with the physical phases of plant physiology. The English edition has been brought up to date by the author's revisions, made late in 1929.

The book is divided into an introduction and eight chapters. In chapter I there is a discussion of those subjects that serve as a basis for chemical plant physiology: colloids, catalysis, enzyme action, velocity of chemical reactions, hydrogen-ion concentration, and related subjects. Chapters II–IV are devoted respectively to photosynthesis, chemosynthesis and nitrogen fixation, and the nutrition of plants by means of prepared organic compounds. Mineral nutrition is considered in chapter V, the metabolism of carbohydrates and proteins in chapter VI, and the fats, lecithins, organic acids, tannins, etc., in chapter VII. The last chapter is devoted to respiration and fermentation.

Taken as a whole, the book is a good summary of the life processes of the plant, considered in their chemical aspects. The researches summarized are mainly German and Russian, including, however, a number of the more important English contributions and some American ones. The translator has, in a degree, made up the lack of adequate references to American work. The book serves as a good source of information concerning Kostychew's own researches. While he has in his investigation emphasized respiration, one is impressed by the breadth of his research interests. There is hardly a phase of plant physiology he has not enriched by original contributions. At the close of many of the sections there are considered the research methods used in that particular phase of the subject. These are considered mainly from the standpoint of the principle involved, although references are given from which the details of the methods may be obtained.—S. V. Eaton.

[1] Kostychew, S., Chemical plant physiology. Translated and edited by C. J. Lyon. pp. xv+497. *figs. 45*. P. Blakiston and Son. Philadelphia. 1931.

Ferns and flowering plants of Hawaii National Park

It is now more than 40 years since HILLEBRAND's *Flora of the Hawaiian Islands* appeared. Meanwhile great advances have been made in the study of the Hawaiian plants, and hundreds of new species have been described. Such workers as C. N. FORBES, J. F. ROCK, and OTTO DEGENER have pushed forward their taxonomic studies with noteworthy zeal. Unfortunately, FORBES is now dead and ROCK has withdrawn to another field. It is all the more gratifying, therefore, to know that DEGENER remains to continue the work. His *Flora Hawaiiensis*, which he has in preparation, bids fair to be a really monumental work in plant taxonomy. His recently issued volume[2] is more popular in its treatments than a conventional flora is apt to be, yet even this smaller work has such a valuable storehouse of taxonomic information that it will prove indispensable to students of Hawaiian plants for years to come. A comprehensive glossary of native works is given. Much of the early history of Hawaiian exploration and discovery has been incorporated. There are various excellent photographs, and these are supplemented with 95 full-page plates, most of them original and of a high degree of excellence. In this way a not inconsiderable number of obscure Hawaiian species have received published illustration for the first time. Various new names which have been found necessary under nomenclatural rules have been omitted, the author preferring to leave such names for publication in his forthcoming *Flora Hawaiiensis*.

Aside from taxonomy, the volume makes a distinct appeal to many people of diverse tastes. The geology of the Hawaiian Islands, the ethnographic relations of the inhabitants, the wars of conquest and defense fought in bygone days, the tribal and family customs which once held sway, the primitive methods of preparing food—these and numerous other subjects receive a treatment that is both authoritative and absorbingly interesting.—E. E. SHERFF.

Physical Properties of Soil

An excellent summary[3] of our knowledge of the physical properties of the soil has been written by KEEN. It is one of the Rothamsted monographs on agricultural science, and meets the need for a book of this kind. As the author points out, it is only in recent years that the subject has been put on a firm scientific basis, yet the treatment of this field in current textbooks of agriculture is based largely on early ideas, many of which recent investigations have shown are erroneous. These investigations are well summarized in the book.

The volume opens with a historical introduction, in which there is treated the relations between the development of our knowledge of the physical properties

[2] DEGENER, OTTO, Illustrated guide to the more common or noteworthy ferns and flowering plants of Hawaii National Park. pp. xv+313. *Frontispiece and pls. 95, figs. 45.* Honolulu Star-Bulletin, Ltd. Honolulu. 1930.

[3] KEEN, B. A., The physical properties of the soil. pp. vi+380. *figs. 93.* Longmans Green Co., New York City. 1931.

of the soil and the development of cultivation implements. Chapter II has to do with the technical analysis of the soil. Chapter III considers the physics controlling the movement of water in the soil. The erroneous nature of the "capillary tube" hypothesis is shown. There follow chapters on soil properties in low moisture conditions, soil and clay pastes, the properties of soil and clay suspensions, soil constants and equilibrium points, physical properties of soil under various conditions, and cultivation and cultivation implements. The last two chapters have to do with factors controlling the temperature and aeration of the soil. The literature is critically summarized, and is presented in a clear, well organized, convincing manner. In common with the other Rothamsted monographs, there is a copious citation of literature. The general bibliography at the end of the book contains 263 titles. In addition there are numerous footnotes throughout the book giving other references.—S. V. EATON.

Elements of plant science

In recent years there have appeared numerous textbooks of botany for use in secondary schools and colleges, all with much similarity of contents. The volume by CHAMBERLAIN[4] is notable because it is unique in several ways. The text, carefully planned, is such a simple, clear, and orderly presentation of the most up-to-date botanical knowledge that even beginners in the subject can easily comprehend it. This clarity of statement and careful selection of material are of course possible because of the vast store of botanical knowledge possessed by the author, combined with more than 40 years of sympathetic contact with students of botany. All of the illustrations are original, have been specially made to fit the text, and are very accurate.

Part I, which can be used by schools without microscopes, takes up the structures in a natural manner: leaf, stem, root, flower, fruit, and seed; followed by a chapter on various important topics, and one on laboratory studies. Part II, devoted to the structure and development of plants, is a presentation of their orderly development, and is illustrated with a wealth of excellent drawings and diagrams accurately fitted to the text. The chapter on laboratory methods is a fitting close to what the reviewer believes is the most admirable textbook of botany he has ever seen for use in secondary schools and colleges.— W. J. G. LAND.

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[4] CHAMBERLAIN, C. J., Elements of plant science. pp. xii+394. *figs. 321.* McGraw-Hill Book Co. New York. 1930.

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